GARY R. HERBERT
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State of Utah

DEPARTMENT OF NATURAL RESOURCES

MICHAEL R. STYLER
Executive Director

Division of Wildlife Resources

GREGORY J. SHEEHAN
Division Director

March 30, 2017

QA/QC Laboratory Accreditation Committee,

I am writing to inform the committee that the Utah Division of Wildlife has submitted their application for Tier I status through the laboratory quality assurance program. Our program director Wade Cavender and laboratory manager Christine Swan, together meet the requirements of the FHL position and are both certified Aquatic Animal Health Inspectors (AAHI). Maria Hansen is filling the position of laboratory bacteriologist and QA/QC manager with oversight from Wade and Christine. Chris Heck and Danielle Van Vliet are serving as the stations virologist and parasitologist, respectively. Todd Jones and Cathryn Smith also assist with basic technician responsibilities with direction from AAHI personnel. In accordance with Tier 1 requirements the following items have been submitted:

1. Complete Tier I application
2. All laboratory protocols/SOP's
3. Laboratory blueprint/schematic
4. Employee CV/resumes
5. Chemical hygiene plan
6. Biosafety requirements
7. Integrated pest management plan (IPM)
8. $500 application fee (submitted to FHS treasurer)

Let us know if we can answer any questions or provide additional information.

Thank you,

Wade

Wade Cavender

Wade Cavender
Fisheries Experiment Station Supervisor
Fish Pathologist, MS
Logan, Utah 84321
Cell: 435-720-2784
wadecavender@utah.gov

ORIGINAL

Fish Health Laboratory Quality Assurance Process

Basic Requirements for Tier I Prequalification

The purpose of the Quality Assurance/Quality Control Program (QA/QC) is to ensure the quality, reproducibility and accuracy of the information and results generated within a fish health laboratory. Inspection and diagnostic protocols provided in updated versions of *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens* (http://www.afs-fhs.org/bluebook/bluebook-index.php) AFS Fish Health Section Bluebook) provide excellent standards by which the laboratory can abide when conducting laboratory assays. Following a carefully written protocol, however, does not in itself assure a quality product. In order for a laboratory to assure quality services it must first establish a system of quality control by which every aspect of laboratory management, structure and upkeep are implemented. The purpose of this incremental accreditation program is to provide a structure by which laboratories providing fish disease diagnostic and inspection services can develop individual, lab-specific programs. This document is designed for adaptation at small laboratories as well as large establishments and will serve to address many of the critical elements provided in the ISO (International Organization for Standardization) and IEC (International Electrochemical Commission) International Standards 17025, as well as those standards published by OIE (Office International de Epizooties).

To implement this process a three tier system has been developed (Tier I Prequalification, Tier II Recognition, Tier III Accreditation). Tier I is the prequalification step which includes identification of key personnel and their qualifications, required laboratory facilities, standard operating procedures and laboratory safety. The objective of Tier I is to demonstrate that a particular laboratory has established a quality system and is suitable to move on to Tier II Recognition. Specific guidelines to assist in meeting Tier I requirements can be found in the AFS Fish Health Section Blue Book, Chapter 3 (*Model Quality Assurance/Quality Control Program for Fish Health Laboratories)*. The prequalification designation associated with Tier 1 is valid for a period of 24 months during which time the laboratory is qualified to apply for Tier II status. If the Tier 1 designation expires then the laboratory will need to re-apply for the prequalification status before moving on to Tier II. Tier II and III are in the developmental stages and will be further defined in the future.

Application procedures:

A. Thoroughly review each category throughout the application. If your laboratory is in compliance with the criteria, check the respective boxes and initial subcategories.

B. Applicants must contact Cathryn Smith (cathrynsmith@utah.gov, 435-752-1066) to receive detailed instructions for submitting application forms, supporting documents and $500.00 non-refundable application fee. Make check payable to FHS/AFS.

C. Submission date for each calendar year will be March 31.

D. Application will be reviewed by the committee and approved/denied by June 30 of each year.

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Tier I Prequalification Application for Fish Health Laboratories

American Fisheries Society/Fish Health Section

Personnel

1. Have you identified a Fish Health Laboratory (FHL) director who has met the following qualifications?
   ◉ Yes ○ No

   The FHL Director shall have overall responsibility for the technical integrity of the tests as well as for interpreting, analyzing, documenting and reporting result. The Director will ensure that:

   Initials

   CS a. *Employees clearly understand the functions which they are to perform, and are properly trained to perform their duties, and that training is documented;*

   CS b. *Any deviations from this QA/QC Program or unforeseen circumstances that may impact the integrity of the tests are corrected and documented, and;*

   CS c. *All test data are accurately and precisely recorded and reported.*

   Required material: Specifically identify the FHL director and attach a resume which includes qualifications, skills, experience and certifications.

2. Have you identified a Quality Assurance Coordinator who has met the following qualifications? (Note: Depending on laboratory personnel, the Director and Coordinator may function in the same position.)
   ◉ Yes ○ No

   The FHL shall have a Quality Assurance Coordinator whose responsibilities include the following:

   Initials

   CS a. *Implementing and monitoring the QA/QC Program*

   CS b. *Implementing all necessary quality controls to ensure the accuracy and precision of reported data.*

   CS c. *Monitoring laboratory practices to verify continuing compliance with policies and procedures.*

   CS d. *Evaluating instrument calibration and maintenance records.*

   CS e. *Ensuring the validation of new technical procedures.*

   CS f. *With the Director, investigating technical problems, proposing remedial actions, and verifying their implementation.*

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CS g. *Providing recommendations for training to improve the quality of laboratory staff.*

CS h. *Proposing corrections and improvements in the QA/QC system.*

Required material: Specifically identify the Quality Assurance Coordinator and attach a resume which includes qualifications, skills, experience and certifications.

3. Have you identified other technical staff members that will participate in developing and implementing the QA/QC program?
   ◉ Yes ○ No

Required material: Specifically identify other technical staff members and responsibilities. Attach a resume which includes qualifications, skills, experience, certifications and any additional good laboratory practice (GLP) training received.

Note: Employee resumes not included in this example to reduce document size

Laboratory Facilities

1. Does your laboratory facility meet the following criteria?
   ◉ Yes ○ No

Initials

CS a. *Laboratories should designate separate areas for administrative activities, fish handling, and laboratory testing.*

CS b. *Each laboratory room has adequate space and safe environmental conditions to perform assigned tasks.*

CS c. *All laboratory space is adequate to maintain equipment, supplies, samples, and chemicals without danger of cross contamination.*

Required material: Provide a blueprint or schematic of laboratory facilities.

2. Do all laboratory equipment and supplies used in your laboratory meet the following requirements?
   ◉ Yes ○ No

Initials

CS a. *All microscopes, balances, pipettes, thermometers, meters, incubators, refrigerators, freezers, hoods, spectrophotometers, and other instruments in use are calibrated and maintained on a routine basis by laboratory staff.*

CS b. *Each item of equipment possesses an inventory number for identification. Records of calibration and maintenance documentation are kept for each instrument and microscope.*

CS c. *Where appropriate maintenance and temperature information is posted on its equipment (hoods, balances, refrigerator/freezers, incubators).*

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CS d. *Defective or suspect equipment is taken out of service until repaired, tested and recalibrated.*

CS e. *Operation manuals and/or Equipment standard operating procedure (SOP) for each piece of laboratory equipment are available.*

CS f. *Equipment used for generating measurements is calibrated and/or standardized according to recommendations provided in Appendix A (Section III, Appendix E within Blue Book).*

Required material: Provide equipment, calibration and maintenance SOP's.

3. Are all reagents and reference stocks maintained under proper storage conditions, labeled and handled appropriately by all laboratory staff according to the following guidelines?
   ◉ Yes ○ No

Initials

CS a. *All reference stocks shall be retained with original labels from the supplier or are labeled by name, date of receipt, chemical abstracts number (CAS) or code number, batch number, expiration date if perishable, and include National Fire Protection Association (NFPA) labels indicating safe use and storage requirements.*

CS b. *Reagents are labeled to indicate identity, contents, titer or concentration, expiration date and safe use and storage requirements (NFPA labels).*

CS c. *Reference stocks and reagents are handled in a manner that precludes the possibility of contamination, deterioration, or damage to the substance.*

CS d. *All reagents, serums, cell lines, and laboratory supplies are of high quality. To ensure quality performance during all laboratory assays, specific product and manufacturer sources are provided where indicated in accepted protocols.*

CS e. *An inventory of all reagents is maintained for the monitoring of expiration dates. Deteriorated or outdated reference stocks and reagents are disposed of properly.*

CS f. *Mixture of substances - When test, control, or reference substances are mixed, the date of preparation, initials of the preparer, and the exact contents of the mixture shall be labeled on the bottle.*

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Chain of Custody/Case Tracking

1. Are all cases/samples submitted to the laboratory and tracked according to the following protocols described below?
   ◉ Yes ○ No

   Initials

   CS a. *All samples are given a case history number as they are received at the laboratory.*

   CS b. *The case history number uniquely identifies the test samples on receipt and tracks the case throughout the laboratory. Upon receipt the case number is assigned and labeled on all sample containers.*

   CS c. *The case history number, along with information pertaining to the specifics of the samples received, is recorded on either a Case History Record (CHR) cover sheet and/or in a Case Report book. The following information is to be included:*
      1) *Case History Number*
      2) *Date of Receipt*
      3) *Date Sample Taken*
      4) *Sample Site (including, where possible, GIS information)*
      5) *Name of Sampler*
      6) *Recorder Initials*
      7) *Species and Age -class of fish*
      8) *Condition of Samples at receipt*

   CS d. *This Case History Record (CHR) cover sheet also contains specific numbers and tissue materials collected for the following lab assays: Bacteriology, virology, parasitology, serology, histology, molecular (PCR) and "other". In addition, any descriptive information received with the samples is attached to the CHR.*

   CS e. *All sample material is assigned a number which corresponds directly with the description recorded in the CHR.*

   CS f. *All tubes, bags, or other sample containers are labeled with pertinent information using chemical resistant markers to allow for accurate tracking through each laboratory area.*

   CS g. *All CHR's are transcribed in ink, and recorded in a manner to ensure the integrity of all samples from collection site to final analysis.*

2. Sample tracking in individual labs

   CS a. *If sample items are sent to an outside laboratory for expert analysis, the transfer of that item is properly entered on the CHR. Results from the outside laboratory are obtained in writing and attached to the CHR.*

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CS b. *Within each laboratory area (bacteriology, virology, etc.), a separate record system is maintained to record samples received into the area, assays requested and performed and results obtained. At completion of all assays, the results are recorded onto the original CHR, and any supporting paperwork attached.*

CS c. *When all assays are completed and results are obtained, the CHR with all necessary attachments is provided to the designated staff member for a case report write-up. All reports refer to the appropriate CHR number, and copies are maintained in laboratory files.*

3. Record Retention

CS a. *Hard copies of records are retained in office files for at least 7 years. This record retention standard also applies to computer records retained on disk.*

CS b. *Equipment logs are maintained for a minimum of two years.*

Required material: Provide example of laboratory case history log and sample tracking forms.

Standard Operating Protocols and Conduct of tests

1. Are all laboratory procedures conducted in accordance with standard operating protocols according to the following guidelines?
   ⦿ Yes ○ No

   Each fish health laboratory shall adhere in strict accordance with the specific protocols depending upon the type of case being conducted.

Initials

CS a. *Fish Health Inspection samples are assayed according to the current edition of Procedures for Aquatic Animal Health Inspections (AFS-Fish Health Section "Bluebook"), and/or other state or provincial regulations, regional fish health compact guidelines, and/or international requirements (OIE) that may apply.*

CS b. *Directors are responsible for approving other protocols prior to their use. Critical protocol deviations must be documented on the CHR with a description of the procedures used, and/or citation from the literature.*

CS c. *Data generated during all tests shall be documented, in ink, and attached to the CHR. Result summaries are entered directly onto the CHR cover sheet.*

CS d. *Pertinent entries are dated and initialed by the employee performing the work.*

CS e. *Any changes to the original entry should not obscure the original entry and the reason for the change should be indicated, dated, and initialed by the employee performing the change.*

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Required material: Copies of all SOPs must be submitted with your application. The recommended format for all laboratory SOP's can be found in Appendix B.

2. Is your laboratory in compliance with the laboratory safety procedures outlined below?
   ◉ Yes ○ No

Initials

CS a. *Fish Health Laboratories working with infectious agents that pose moderate hazards to personnel and the environment must comply with national and local standards of health and safety at or equivalent to BioSafety Level II Containment. Laboratory personnel need not confine established cultures with low aerosol potential to an approved safety cabinet. Laboratory biosafety level 2 criteria are outlined in Biosafety in Microbiological and Biomedical Laboratories, 1999 U.S. Department of Health and Human Services, Public Health Services, Center for Disease Control, and National Institutes of Health, 4th edition, U.S. Government Printing Office, Washington D.C.* (http://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf#x2013).

CS b. *Copies of MSDS's (Material Safety Data Sheets) for all chemicals and reagents in the laboratory are kept on file (hardcopy, digital or web-based) and within easy access and viewing for all personnel. All personnel are to follow safety precautions published within MSDS's for each reagent used at the laboratory.*

CS c. *Necessary personal protective equipment and training in equipment use and safety is provided and documented for all laboratory personnel.*

CS d. *Fish Health Labs should have a "Safety and Chemical Hygiene Plan" as seen in the following (https://www.osha.gov/Publications/laboratory/OSHAfactsheet-laboratory-safety-chemical-hygiene-plan.pdf). All laboratory personnel are to utilize equipment and reagents in compliance with this plan.*

Required material: Attach a copy of your Safety and Chemical Hygiene Plan and a summary of Biosafety II requirements as described above. Include any documented training for all employees.

Please Sign and Date Prior to Submission.

I do hereby attest that I have reviewed all the information and responses contained within this application, and that they are accurate to the best of my knowledge.

Signature________________ **Date** 3/28/17

ORIGINAL

Fisheries Experiment Station
Quality Assurance and Quality Control Manual
Initiated Spring 2017

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FES Blueprint

Personnel CV

FES Chemical Hygiene Plan

Biosafety Requirements

Integrated Pest Management Plan

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Definition and Use of the Standard Operating Procedures (SOPs)**	
SOP #: 101	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE

This document describes the definition and use of standard operating procedures (SOPs) within the Fisheries Experiment Station (FES) program.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. Definition of a SOP:
   - The SOP is a written "how-to" document that personnel use to perform procedures that are routine, standardized and for which modification must be justified and approved.
     - To be considered effective, a SOP must have the approval and signature of the FES management (typically the Fish Health Specialist or Station Director).
   - Using SOPs helps to ensure the quality and integrity of data.
   - The SOP provides a basis for uniformity and accountability and can also be used for training and guidance.
2. FES SOPs are categorized by purpose:
   - Bacteriology SOPs describe procedures for sample collection and handling, diagnostic assays, etc., for the detection and identification of bacteria.
   - Field Sampling SOPs describe procedures for field sampling, such as collection of tissue for subsequent use in diagnostic assays.
   - General SOPs describe standardized procedures (e.g., for quality assurance, animal care, data acquisition and security).
   - Instrumentation SOPs outline the standard steps to calibrate, operate and maintain laboratory and field instruments.
   - Media SOPs describe procedures for the preparation of media, reagents and related materials used in any of the SOPs.
   - Parasitology SOPs describe procedures for sample collection and handling, diagnostic assays, etc., for the detection and identification of parasites.
   - Molecular Techniques SOPs describe molecular procedures, including DNA extraction, PCR and gel electrophoresis.
   - Virology SOPs describe procedures for sample collection and handling, diagnostic assays, etc., for the detection and identification of viruses, including cell culture.

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- Signed, original SOPs are considered the definitive document; personnel may work from physical or electronic copies of the signed originals. Signed originals, including superseded versions, are maintained in an archive at the FES.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 102, Developing Standard Operating Procedures

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Developing Standard Operating Procedures (SOPs)**	
SOP #: 102	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE

This document was developed to ensure that all standard operating procedures (SOPs) created within the Fisheries Experiment Station (FES) are consistent with accepted practices, quality assurance/quality control standards, and any other existing SOPs.

B. DEFINITIONS

1. FES management: typically Fish Health Specialist or Station Director

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. Development of SOPs:
   - SOPs can be drafted by any FES employee.
   - Draft SOPs are must be created using a previously approved and standardized format.
   - Documents are reviewed for accuracy and adherence to accepted practices and approved by (i.e., signed and dated) by FES management.
   - Approved SOPs will be stamped "ORIGINAL" in red ink and filed in a secured file cabinet. The filed "ORIGINAL" is considered to be the only official approved SOP.
   - The title and number of approved SOPs will be listed as active on a master index, to be filed with the "ORIGINAL" SOPs.
   - A copy of all active working SOPs will be stored in the laboratory and the office of the FES management.
2. Revision of SOPs:
   - Any SOP may be revised by any FES employee.
   - SOPs must be revised if equipment or instrumentation are replaced, if methods are revised or if testing requirements have changed.
   - The new, revised SOP will be marked "ORIGINAL" in ink and handled according to procedures described above. The SOP being replaced will be marked "SUPERSEDED" and placed in the appropriate section of the SOP files.
     - A red-line copy (i.e., track changes) of the revised SOP, detailing the changes made (e.g., additions and deletions to the SOP) will be filed with the new, revised "ORIGINAL" SOP.
   - On the master index, the revised version is logged on the active SOP list and the old version is logged as superseded.
3. Inactivation/Deletion of SOPs:
   - SOPs can be recommended for deletion or inactivation by any FES employee.

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  - Inactivation is recommended for SOPs regarding practices that no longer commonly apply to the FES (e.g., for old equipment that functions but is no longer used routinely; for techniques that are considered appropriate but no longer routine).
  - Deletion is recommended for SOPs that no longer apply to the FES program (e.g., for discarded equipment, for techniques no longer considered appropriate or are inapplicable) or SOPs that have been inactive for at least 3 years.
  - SOPs recommended for deletion or inactivation will be reviewed by FES management and the SOP will be deleted if necessary.
  - The SOP will be marked "DELETED" or "INACTIVATED" in ink and placed in the appropriate section of the SOP files.
  - Inactivated SOPs shall be retained indefinitely.
  - It is not necessary to retain deleted SOP files (though the SOP should still be logged as deleted on the master index).
  - On the master index, the SOP is removed from the active SOP list and logged as deleted or inactivated.

4. Reactivation of SOPs:
  - Inactive SOPs can be recommended for reactivation by any FES employee. Deleted SOPs cannot be reactivated.
  - If an active SOP is desired, the information must be developed and evaluated as a new SOP.
  - An SOP recommended for reactivation will be reviewed by FES management and the SOP will be reactivated if necessary.
  - The SOP will be marked "REACTIVATED" in ink and returned to the appropriate section of the SOP files.
  - On the master index, the SOP is removed from the inactive SOP list and logged as an active SOP.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 101, Definition and Use of the Standard Operating Procedures (SOPs)

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Quality Assurance and Quality Control (QA/QC) Administration**	
SOP #: 103	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the administrative activities and responsibilities intended to ensure the quality, reproducibility and accuracy of the information and results generated by the Fisheries Experiment Station (FES). The activities and responsibilities described herein were adapted from the model Quality Assurance and Quality Control (QA/QC) documentation cited below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECATIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. Management Responsibilities:
   - FES shall have a Fish Laboratory Supervisor (hereafter “Supervisor”).
   - The FES supervisor shall have overall responsibility for the technical integrity of the tests as well as for interpreting, analyzing, documenting and reporting results. The supervisor will ensure that:
     - Employees clearly understand the functions which they are to perform and are properly trained to perform their duties and that training is documented[1].
     - Any deviations from this or other QA/QC SOPs or other factors affecting the integrity of FES tests or results are documented and corrected;
     - All test data are accurately and precisely recorded[2], reported and archived[3].
   - The supervisor shall act as the quality assurance coordinator for the FES with the following responsibilities:
     - To be accountable for QA/QC concerns of the FES to the NRDWR aquatics chief and director, entities with shared jurisdictional authority and funding entities.
     - Implementing and monitoring of QA/QC-related SOPs.
     - Implementing all necessary quality controls to ensure the accuracy and precision of reported data.
     - Monitoring laboratory practices to verify continuing compliance with policies and procedures.
     - Evaluating instrument calibration and maintenance records[4].
     - Ensuring the validation of new technical procedures.
     - Investigating technical problems, proposing remedial actions and verifying their implementation.

ORIGINAL

    - Providing recommendations for training to improve the quality of laboratory staff.
    - Proposing corrections and improvements to FES procedures, including QA/QC-related activities and SOPs[5].
    - Cooperate with and accompany teams conducting external and/or internal QA/QC audits of the facility.
2. Personnel Responsibilities:
    - FES shall maintain an adequate number of employees for all functions. All employees shall possess the necessary education, training, technical knowledge, skill and experience for the work they do.
    - All employees will act in a manner consistent with all NRDWR policies and procedures and shall be free from pressure or inducements which might adversely influence their judgment or the results of their work.
    - Each employee shall be responsible for monitoring each test he/she is conducting to ensure that facilities, equipment, practices and records-keeping conform to this and other FES SOPs.
    - Each employee shall take the necessary precautions to avoid contamination of test, control and reference substances.
    - Each employee shall follow pertinent safety and health regulations, NRDWR policies and procedures, SOPs, manufacturer's instructions, etc., regarding workplace safety; equipment operation; handling, storage and disposal of hazardous materials, etc.[6, 7, 8, 9].
3. Records of Personnel Qualifications:
    - A technical qualifications file shall be maintained for each laboratory staff member, including the following:
        - A resume or curriculum vita describing qualifications, skills, experience and certifications.
        - References to all training classes, seminars, short courses and conferences attended.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 104, Position Descriptions, Curriculum Vitae/Resumes and Training Records
2. SOP# 105, Recording Data
3. SOP# 107, Maintaining Records and Archives
4. SOP# 202, General Use and Maintenance of Equipment
5. SOP# 102, Developing Standard Operating Procedures (SOPs)
6. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
7. SOP# 201, Personal Protective Equipment
8. SOP# 208, Disposal of Infectious Materials
9. SOP# 209, Disposal of Hazardous Wastes
10. AFS-FHS (American Fisheries Society-Fish Health Section) FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Model Quality Assurance/Quality Control Program for Fish Health Laboratories. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

SOP Title: **Position Descriptions, Curriculum Vitae/Resumes and Training Records**	
SOP #: 104	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to ensure that all Fisheries Experiment Station (FES) personnel are adequately trained to perform their duties and that such training is documented.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. Position (job) descriptions:
    - Position descriptions, including a description of duties, necessary skills, etc., must be on file for all permanent FES personnel.
    - Position descriptions must be updated within 3 months of changes being approved by UNRDWR Human Resources; descriptions of new positions must be filed within 3 months of hiring.
    - Position descriptions are encouraged, but not required for temporary FES personnel or non-FES cooperators.
2. Individual training records:
    - Curriculum vitae/resumes must be on file for all permanent FES personnel.
    - Curriculum vitae/resumes should be updated within 3 months of hiring or any significant change in status (e.g., completion of a degree or certification, promotion to a new position) and annually thereafter.
    - Curriculum vitae/resumes should include descriptions of formal education/training, continuing education activities, attendance at professional conferences/workshops and any other information pertinent to the employee's competence in fulfilling duties described in their position description.
    - Curriculum vitae/resumes are encouraged, but not required for temporary FES personnel or non-UNRDWR cooperators.
    - All FES personnel must complete mandatory training related to FES operations.
    - Safety related training should be completed within 3 months of hiring.
    - Complete employee safety training program.
    - Review SDSs for all materials encountered in the course of the employee's duties.
    - FES SOPs should be reviewed within 3 months of hiring and annually thereafter.
    - New SOPs should be reviewed within 3 months of adoption.

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- All FES personnel must complete mandatory training required of UNRDWR personnel (e.g., sexual harassment prevention, ethics).
- Training should be documented on the employee's training records; additionally, such training may be documented on the employee's curriculum vita/resume.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. Electronic curriculum vitae/resumes of all employees.
2. Electronic training records of all employees.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Recording Data**	
SOP #: 105	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to ensure data is properly recorded and uploaded.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. All raw data is to be recorded:
   - Directly.
   - Promptly.
   - Legibly.
   - In ink.
   - Onto appropriate data collection sheets[1] .
2. All data entries should be immediately dated and signed (or initialed).
3. Original data entries are to be maintained, regardless of their form (e.g., datasheets splashed with coffee, numbers written on a napkin) and given to FES management (i.e., Laboratory Supervisor).
4. If true and exact copies of data are to be made (i.e., photocopies, photographs), they must be labeled as such, dated and initialed by the individual making the copy. The location of the original source material is also to be documented on the copy.
5. Data must be uploaded to the appropriate electronic source as soon as possible.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 106, Chain of Custody and Case Tracking

I. REVISION HISTORY

1. Original

ORIGINAL

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Chain of Custody and Case Tracking**	
SOP #: 106	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to ensure all samples received for aquatic animal health testing are recorded in the case log book upon receipt, distributed to the appropriate laboratory section for examination and final results are accurately documented, reported and archived.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Case log data sheet (Appendix A)
2. Inspection inventory request form (Appendix B)
3. Pathogen survey request form (Appendix C)
4. Diagnostic collection form (Appendix D)
5. *Myxobolus cerebralis* PTD sample tracking sheet (Appendix E)
6. *Myxobolus cerebralis* PCR sample tracking (Appendix F)
7. *Renibacterium salmoninarum* DFAT sample tracking sheet (Appendix G)
8. Diagnostic case bacterial growth tracking sheet (Appendix H)
9. *Flavobacterium psychrophilum* DFAT sample tracking sheet (Appendix I)
10. Antibiotic sensitivity data sheet (Appendix J)
11. Virology sample tracking sheet (Appendix K)
12. Sharpie and ink pen

E. PROCEDURES

1. Case History Log/Collection Forms:
   - Prior to collection all samples are given a case history number which is recorded in the case log data sheet (Appendix A).
   - The case history number uniquely identifies the test samples and tracks the case throughout the laboratory.
   - The appropriate collection form (Appendix B-D) must be completed and at a minimum should include the following:
     - Case history number
     - Date samples collected
     - Date of receipt
     - Sample site (including, where possible, GIS information)
     - Fish species and age class

ORIGINAL

- Any descriptive information received with the samples should be included in the comments section of each collection form.
- An electronic copy of the collection form will be stored in the appropriate folder on the G: drive and a hard copy of the inspection, diagnostic case or pathogen survey report will be stored in the main office.
- Upon receipt, all tubes, bags or other sample containers are to be labeled with a chemical resistant laboratory marker with the assigned case number to allow for accurate tracking of samples through each laboratory area.
- All case history information is transcribed in ink and recorded in a manner to ensure the identity and integrity of all samples from collection site to final analysis.

2. Sample tracking within individual laboratory sections:
   - Distribute samples to their correct storage location.
     - Kidney samples for BKD testing are stored in the -80°C freezer located in the main laboratory (Appendix G).
     - Virology samples are stored in the sample refrigerator in the virology laboratory (Appendix K).
     - Fresh cranial material for PTD analysis is stored in the sample refrigerator located within the main laboratory (Appendix E).
     - Cranial material for PCR analysis is stored in the -20°C sample freezer located in the FES conference room (Appendix F).
     - Diagnostic samples should remain in the coolers in which they were delivered and be temporarily stored in the sample refrigerator located in the main laboratory (Appendix H-J).
   - Within each laboratory section (bacteriology, virology, parasitology, etc.) a separate record system is maintained to document samples received into the area.
   - If sample items are sent to an outside laboratory for analysis, the transfer of that item is properly recorded in the case history log.
   - When all assays are completed, results from each section are provided to the appropriate staff member in order for that person to create and submit a final report for each case.
3. Record retention:
   - Hard copies of records are retained in office files for at least 7 years. This record retention standard also applies to all electronic records.

ORIGINAL

F. QUALITY CONTROL

- Case history for each sample collection must be recorded immediately and accurately in order to properly document the chain of custody throughout the laboratory.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. American Fisheries Society-Fish Health Section (AFS-FHS) Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens (most recent edition). Section 3. QA/QC Model for Fish Health Labs. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php .
2. Colorado Parks and Wildlife. Aquatic Animal Health Laboratory Procedures Manual. 2016.
3. Idaho Fish and Game. Aquatic Animal Health Laboratory Procedures Manual. 2016.

I. REVISION HISTORY

1. Original

J. APPENDICES

- 106.A Appendix: Case Log Data Sheet
- 106.B Appendix: Inspection Inventory Request Form
- 106.C Appendix: Pathogen Survey Request Form
- 106.D Appendix: Diagnostic Form
- 106.E Appendix: *Myxobolus cerebralis* PTD Sample Tracking Sheet
- 106.F Appendix: *Myxobolus cerebralis* PCR Sample Tracking Sheet
- 106.G Appendix: *Renibacterium salmoninarum* DFAT Sample Tracking Sheet
- 106.H Appendix: Diagnostic Case Bacterial Growth Tracking Sheet
- 106.I Appendix: *Flavobacterium psychrophilum* DFAT Sample Tracking Sheet
- 106.J Appendix: Antibiotic Sensistivity Data Sheet
- 106.K Appendix: Virology Sample Tracking Sheet

ORIGINAL

FISHERIES EXPERIMENT STATION
2007 CASE LIST

Case Number	Location	Case Type					Date Collected	Date Received	Initials	Comments
		AI	Supp	Diag.	Survey	Res.				
XX-001										
XX-002										
XX-003										
XX-004										
XX-005										
XX-006										
XX-007										
XX-008										
XX-009										
XX-010										
XX-011										
XX-012										
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XX-026										
XX-027										
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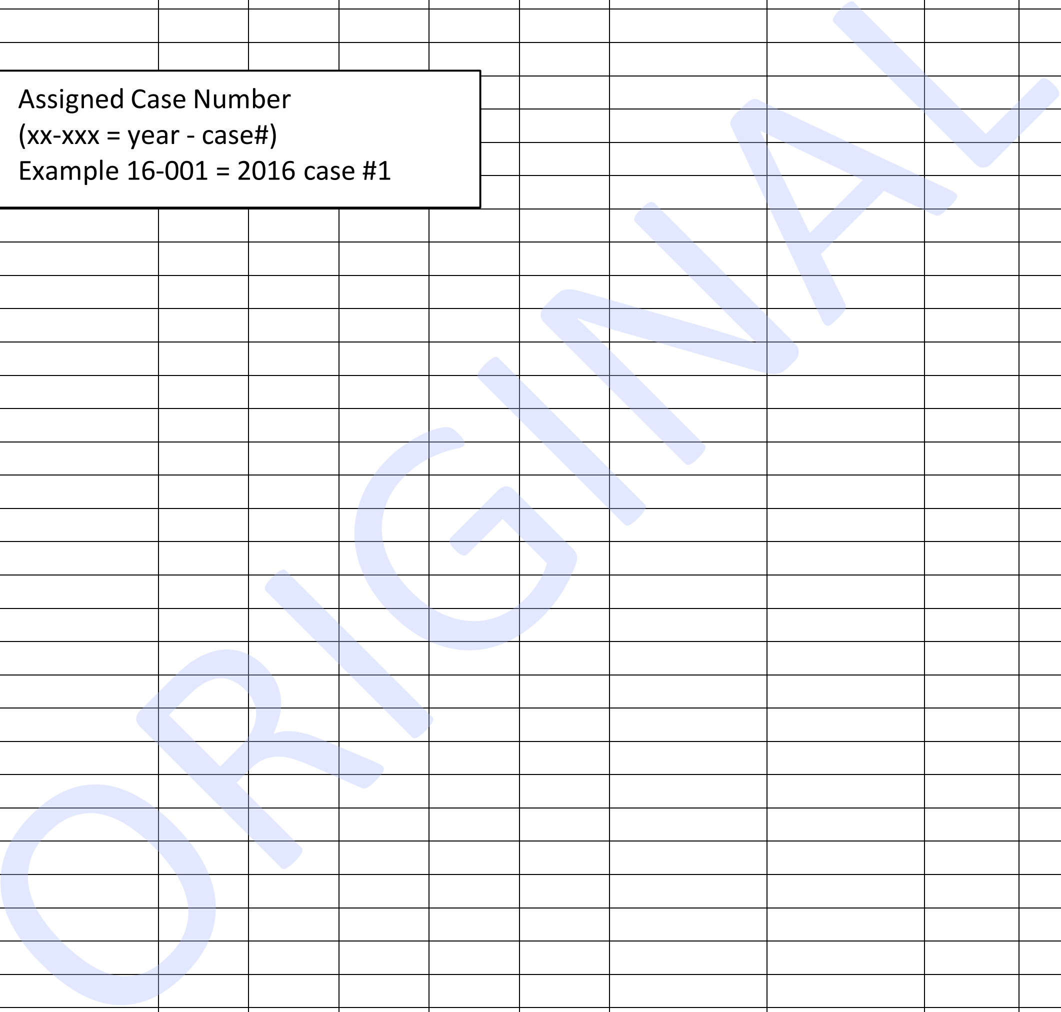
XX-030										
XX-031										
XX-032										
XX-033										
XX-034										
XX-035										



FISHERIES EXPERIMENT STATION
2007 CASE LIST

Case Number	Location	Case Type					Date Collected	Date Received	Initials	Comments
		AI	Supp	Diag.	Survey	Res.				
XX-036										
XX-037										
XX-038										
XX-039										
XX-040										
XX-041										
XX-042										
XX-043										
XX-044										
XX-045										
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XX-067										
XX-068										
XX-069										
XX-070										

ORIGINAL

FISHERIES EXPERIMENT STATION
2007 CASE LIST

Case Number	Location	Case Type					Date Collected	Date Received	Initials	Comments
XX-071										
XX-072										
Case Number	**Location**	**Case Type**					**Date Collected**	**Date Received**	**Initials**	**Comments**
		AI	**Supp**	**Diag.**	**Survey**	**Res.**				
XX-073										
XX-074										
XX-075										
XX-076										
XX-077										
XX-078										
XX-079										
XX-080										
XX-081										
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XX-101										
XX-102										
XX-103										
XX-104										
XX-105										

ORIGINAL

FISHERIES EXPERIMENT STATION
2007 CASE LIST

Case Number	Location	Case Type					Date Collected	Date Received	Initials	Comments
		AI	Supp	Diag.	Survey	Res.				
XX-106										
XX-107										
XX-108										
XX-109										
XX-110										
XX-111										
XX-112										
XX-113										
XX-114										
XX-115										
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XX-134										
XX-135										
XX-136										
XX-137										
XX-138										
XX-139										
XX-140										

ORIGINAL

FISHERIES EXPERIMENT STATION
2007 CASE LIST

XX-141										
XX-142										

ORIGINAL

UTAH DIVISION OF WILDLIFE RESOURCES

FISH HEALTH COLLECTION REPORT

FES Case Number:

Name of Fish Source:	Blank	Name of Owner or Manager:	Blank	Collection Date:	
Address or Location:	Blank	Telephone Number:	Blank		
	Blank	Testing Laboratory:	Fisheries Experiment Station	Collector(s):	
UTM North	0		1465 West 200 North		
UTM East	0		Logan, UT 84321		

FISH EXAMINED					Number of Samples							
FES #	Species[1]	Age/Length[2]	# in Lot	Hatchery Lot # /Obtained as Eggs (E) or Fish (F)	OF	K/S	WV	WF	BKD	MC	AT	Remarks

OF = ovarian fluid; K/S = kidney/spleen; WV = whole viscera; WF = whole fish; BKD = kidney; MC = *M. cerebralis;* AT = Asian Tapeworm

1 Use standard abbreviations (see species codes worksheet)

2 For hatchery fish, give age in months and length in inches.

The biological samples above and/or on additional pages were collected from fish at the above named facility on the date and by the collector(s) listed, with my approval and knowledge, to be transported to the Fisheries Experiment Station for the purpose of pathogen analysis.

Hatchery Owner or Agent

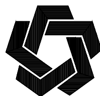
Type of Inspection:

- Annual Health Inspection ☑
- Supplemental Inspection ☐
- Diagnostic ☐
- Research ☐

The biological samples listed above were collected under my supervision at the site specified for pathogen analysis at the laboratory(s) specified.

Fish Health Inspector In Charge

ORIGINAL



PATHOGEN SURVEILLANCE COLLECTION FORM

UTAH DIVISION OF WILDLIFE RESOURCES
Fisheries Experiment Station, 1465 West 200 North, Logan, UT 84321 (435) 752-1066

Site:	**Date Shipped**:
Region:	
Mailing Address:	

County:	**Locale**:
Immediate Drainage:	**UTM E**:
Secondary Drainage:	**UTM N**:
Major Drainage:	**USGS Hydrologic Unit**:

*Note: Please separate lots by species. Collections from different sites **MUST** be entered on separate collection forms. Place this collection form in a watertight Ziploc bag and submit along with the samples. Samples submitted without a signed collection form or accurate UTMs will NOT be processed unless agreed upon beforehand. Contact FES regarding any questions prior to any deviation from the attached sampling protocol.*

FES use only		**Species**	**Age Class**[a]	**Number of Samples**	**Sample Type**[b]	**Date Collected**	**Remarks**
Lot	**Results**						

[a]AGE CLASS (Fish):	**F=Fry**	**F=Fingerlng**	**A=Adult**	**B=Adult Brood**
[a]AGE CLASS (Amphibian):	**T=Tadpole**	**SA=Subadult**	**A=Adult**	
[b]SAMPLE TYPE:	**T=Tissue**	**S=Swab**		

Method of Collection: Electrofishing ☐ Net ☐ Angling ☐ Other________________________

Purpose of Collection:____

____

Collector Signature:____

FES USE ONLY

FES Case Number:	
Condition of Samples Upon Receipt:	
Lab Used For Analysis:	
Number of Samples Tested:	

	Date	**Initial**
Date Received at FES:		
Date Processed:		
Date Entered into Database:		
Sample Disposal Method and Date:		

Updated 12/1/2008

ORIGINAL

Sampling for *Myxobolus cerebralis*

- Collecting samples for routine surveillance
  - Collect a representative sample from each site (e.g. 60 fish for a health inspection or 30 fish for the WD Survey) and group fish by species.
  - Remove heads and double bag in a water-tight ziplock container. *Exception:* if more than one strain of fish are represented (e.g. rainbow trout, brown trout and whitefish), label and bag heads according to strain for each site.
- Collecting samples to identify infection prevalence vs. routine surveillance
  - Contact personnel at FES prior to sample collection to identify specific requirements.
  - Collect a representative sample of fish (in most cases 60 fish).
  - When testing for infection prevalence, every effort should be made to prevent cross-contamination between samples. Aseptically remove heads by sterilizing cutting tool between each fish. Sterilization can be accomplished by first removing tissue from cutting tool and then following one of three sterilization or cross-contamination prevention techniques: 1) wipe the blade with a paper towel, dip in alcohol and flame 2) wipe with store bought unconcentrated bleach or bleach wipe, or 3) change the cutting blade between each sample
  - Securely wrap and identify each head separately (foil, saran-wrap, or zip-locks can be used) and then place all heads into one or several large zip-lock bags.
- Transport and storage
  - Samples can be kept on ice and immediately sent to FES by next day delivery or frozen and forwarded at a later date, not to exceed 6 months.
- Labeling and paperwork
  - Label zip lock bags with the name from each collection site.
  - Completely fill out the collection form and send a copy along with samples collected from each site.
    - Lump entries by species (e.g. Species = RT, Number of Samples = 30; Species = CTCR, Number of Samples = 30; etc).
    - State the purpose for each collection.
    - If entries exceed space provide on this form attach a computer generated list which includes additional information.
- **QUALITY ASSURANCE/QUALITY CONTROL**
  - When handling animals between sites, use a fresh (previously unused) pair of gloves. If gloves are not available, thoroughly wash hands and equipment between sites.
  - Use a clean dissection surface and separate storage container for each animal.

Sampling for *Batrachochytrium dendrobatidis*

- Collecting samples using a Cotton Swab
  - Swab comprehensively (repeat 5-10 times) on the underside of feet, legs and drink patch. For consistency, swab every animal in the same manner. Place sample(s) into a clean (unused) collection container.
  - Samples should be kept cool (0 ºC-4ºC) and sent to FES immediately following each collection.
- Collecting Tissue
  - Collect tissue with a clean scalpel or toothpick. REFER to details below regarding QA/QC on how to maintain sample integrity.
  - Place each sample into a clean (unused) collection container and either (a) place at 4ºC or colder or (b) add 70% ethanol at a ratio of 10:1 and place at 4ºC or colder until sent to FES.
- Transport and storage
  - Samples should be kept cool (0 ºC-4ºC) and sent to FES immediately following each collection.
  - Long term storage should include refrigeration at 0 ºC-4ºC to inhibit growth of other organisms such as bacteria that may interfere with a PCR reaction
- Labeling and paperwork
  - Ensure each animal, tissue, or representative swab is individually labeled.
  - Completely fill out the collection form.
    - Entries can be made individually or grouped by species.
    - State the purpose for each collection.
    - If entries exceed space provide, attach a computer generated list which includes additional information.
- **QUALITY ASSURANCE/QUALITY CONTROL AMONG SAMPLES COLLECTED FOR PCR ANALYSIS (Deviation from these basic conditions can compromise data between sites and between animals.)**
  - When handling animals between sites, use a fresh (previously unused) pair of gloves. If gloves are not available, thoroughly wash hands and equipment between sites.
  - Use a fresh scalpel/tooth pick/swab for each animal.
  - Use only recommended consumables (e.g. MW 100-100 cotton swabs sourced from Biomirieux Aust.).
  - Use a clean dissection surface and separate storage container for each animal.
  - Use freshly prepared storage media (e.g. ethanol) that has been prepared in a Bd-free area.

ORIGINAL

UTAH DIVISION OF WILDLIFE RESOURCES - FISHERIES EXPERIMENT STATION
FISH DISEASE DIAGNOSTIC LABORATORY/SAMPLE SUBMISSION FORM

FES Case Number:	
Date Received:	
Diagnostician:	

SOURCE INFORMATION

Facility Name:	Choose Location
Address:	Blank
City, State, & Zip:	Blank
Phone:	Blank
Submitted by:	Brandon Ivory

SAMPLE SUBMISSION

Species/Strain/Lot Number:	
ation of Raceway/Tanks Affected:	
Average Length:	
Number of fish per pound	
Total # of Fish in Lot:	
Number of fish in affected unit	
Biomass	#DIV/0!
Age (months):	
Flow Index:	
Density Index:	
Current Proposed Stocking Date:	
Proposed Stocking Location(s)	

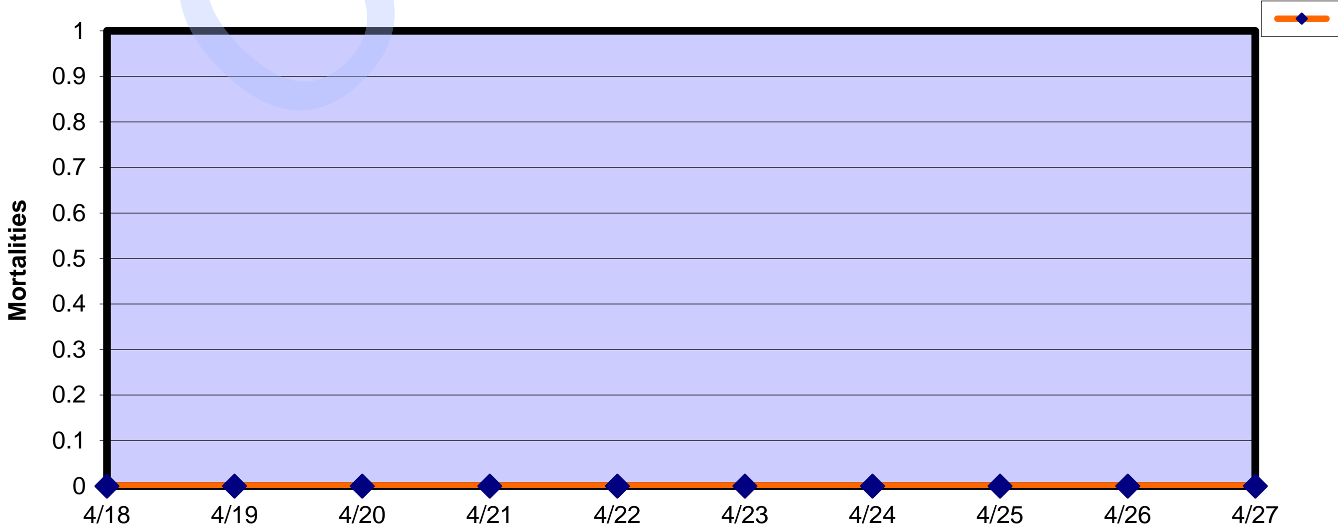
MORTALITY INFORMATION

Date of First Mortality: 4/18/16

4/18	4/19	4/20	4/21	4/22	4/23	4/24	4/25	4/26	4/27

Total Mortality to Date:	0
Percentage of Total:	#DIV/0!

ORIGINAL



WATER QUALITY INFORMATION

Water Temperature: F° °C
Dissolved Oxygen: Head Tail
Total Ammonia:
pH:
Gas Saturation:
Other:

Oxygen Stress? (date):
Other Possible Stresses:

Recent Treatments to Fish or Water (Y/N):
If yes, give type, amount, date, results:

CLINICAL SIGNS/FINDINGS OBSERVED BY CULTURISTS

Location of sick fish in raceway/tank:
Swimming Action:
Flashing? (Y/N)
External body appearance/color:
Gill Action/Appearance:
Feeding Behavior/Appetite:
Observed Lesions:
Microscope Findings:
Other Findings:

Remarks:

DIET

Manufacturer:
Floating or Sinking?
Size of Pellet:
Storage Location:
Condition of Feed:
% Body Weight Recommended:
% Body Weight Actually Fed:
Times/day to feed:

ORIGINAL

Myxobolus cerebralis Pepsin Trypsin Digestion Tracking Sheet

Sample Site:	
Date Collected:	
Date Received in Lab:	
Sample Condition:	

Case Number:	
Received From:	
Sample Type:	
Received By:	

Pepsin Trypsin Digestion (PTD)

Sample Processing		
Cooking Date:	Lot #	Comments/Tech
Defleshing Date(s):	Lot #	Comments/Tech
Initial Pepsin Date:	Lot #	Comments/Tech
Additional Pepsin Date(s):	Lot #	Comments/Tech
Trypsin Date:	Lot #	Comments/Tech

Slide Processing		
Date Slides Made:	Lot #	Comments/Tech
Date Slides Fixed:	Lot #	Comments/Tech
Date Slides Stained:	Lot #	Comments/Tech

Slides Read		
Date	Lot #	Comments/Tech

Data Entered		
Date	Lot #	Comments/Tech

ORIGINAL

Myxobolus cerebralis PCR Tracking Sheet

Sample Site:	
Date Collected:	
Date Received in Lab:	
Sample Condition:	

Case Number:	
Sample Type:	
Received From:	
Received By:	

PCR

Sample Processing		
Date Frozen:	Lot #	Comments/Tech
Date Thawed, Punched & Digested:	Lot #	Comments/Tech
Date Extracted:	Lot #	Comments/Tech

PCR		
Date of PCR:	Lot #	Comments/Tech
Date of Gel:	Lot #	Comments/Tech

Data Entered		
Date	Lot #	Comments/Tech

ORIGINAL

Renibacterium salmoninarum DFAT Tracking Sheet

Sample Site:	
Date Collected:	
Date Received in Lab:	
Sample Condition:	

Case Number:	
Sample Type:	
Received From:	
Received By:	

Sample Processing		
Date Frozen:	Lot #	Comments/Tech
Date Thawed:	Lot #	Comments/Tech

DFAT Stain Dilution		DFAT Stain Lot #	
Pos. Control Dilution		Pos. Control Lot #	

Slide Processing		
Date Slides Made	Lot #	Comments/Tech
Date Slides Fixed	Lot #	Comments/Tech
Date Slides Stained	Lot #	Comments/Tech

Slides Read		
Date	Lot #	Comments/Tech

Data Entered		
Date	Lot #	Comments/Tech

ORIGINAL

Diagnostics: Bacterial Growth Tracking Sheet

Sample Site:	
Date Collected:	
Received From	
Fish Examined	

Case Number:	
Date Received in Lab:	
Received By	
Sample Condition	

Sample Processing		
Date Cultured	Tissue Cultured	Media

Date	Growth	Comments/Tech

Diagnostic Procedures/Tests		
Date	Test/Stain	Comments/Tech

ORIGINAL

Flavobacterium psychrophilum Tracking Sheet

Sample Site:	
Date Collected:	
Date Received in Lab:	
Sample Condition	

Case Number:	
Sample Type	
Received From	
Received By	

Sample Processing		
Date Frozen:	Lot #	Comments/Tech
Date Thawed:	Lot #	Comments/Tech

DFAT Stain Dilution		DFAT Stain Lot #	
Pos. Control Dilution		Pos. Control Lot #	

Slide Processing		
Date Slides Made	Lot #	Comments/Tech
Date Slides Fixed	Lot #	Comments/Tech
Date Slides Stained	Lot #	Comments/Tech

Slides Read		
Date	Lot #	Comments/Tech

Data Entered		
Date	Lot #	Comments/Tech

ORIGINAL

Case NO

FES Bacteriology Worksheet

NG = no growth
disc = discard
SC = subculture

DATE	ORGAN	MEDIA	Growth Observed (Days)						
			1	2	3	4	5	6	7
		TYES							
		TSA							

DATE	Incubation Temperature (Celsius)		
	Gram Stain		
	Morphology		
		Colony	
		cell	
	Oxidase		
	Motility		

Antibiotic Sensitivity

R = Resistant
I = Intermediate
S = Sensitive

Media			Radius	Result	Tissue/organ
TYES	Oxytetracycline		0	R	
	Florfenicol		0	R	
	Penicillin		0	R	
	Erythromycin		0	R	

			Radius	Result	Tissue/organ
TSA	Oxytetracycline		0	R	
	Florfenicol		0	R	
	Penicillin		0	R	
	Erythromycin		0	R	

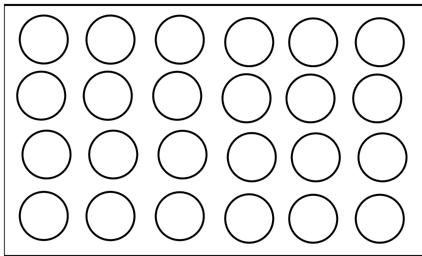
PCR:	

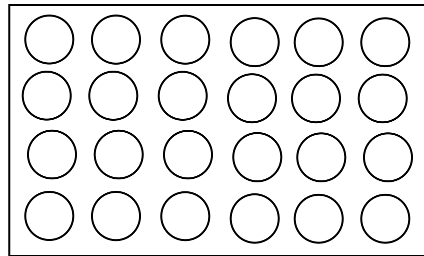
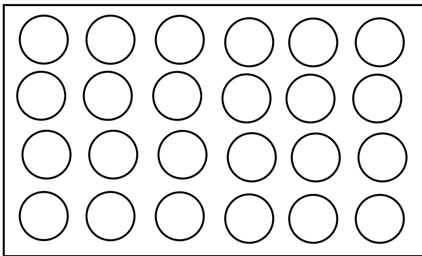
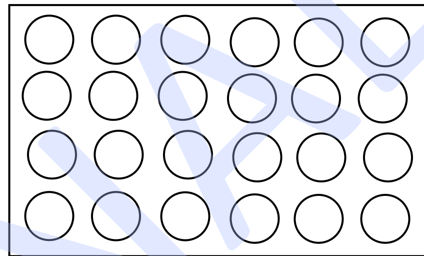
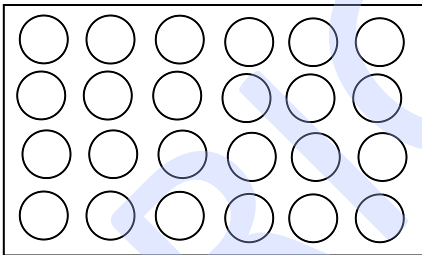
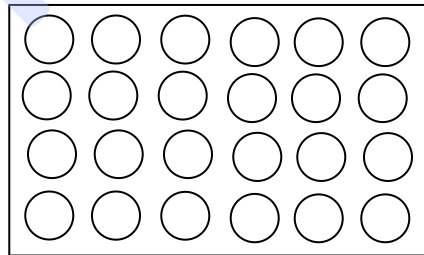
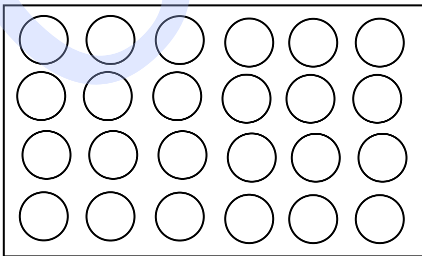
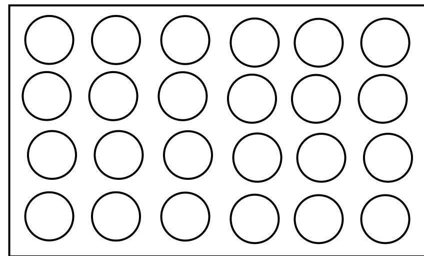
Biochemical Tests (API)	
20 NE	
20E	
ZYM	
NFT	

Other:

Identification:

ORIGINAL



ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Maintaining Records and Archives**	
SOP #: 107	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Administration	Date Next Review Due: 3/30/2020

A. PURPOSE
The purpose of this standard operating procedure (SOP) is to describe the methods the Fisheries Experiment Station (FES) uses to maintain records and archives.

B. DEFINITIONS
1. N/A

C. SAFETY PRECAUTIONS
- N/A

D. EQUIPMENT AND MATERIALS REQUIRED
1. N/A

E. PROCEDURES
1. Records, samples, etc., shall be stored in an orderly fashion to permit expedient retrieval.
2. Conditions of storage shall minimize loss and/or deterioration of the materials (e.g., storage in limited-access, temperature controlled areas; sensitive materials stored in secured, fire-proof units).
3. Electronic records shall be similarly maintained to permit expedient retrieval and to prevent loss and/or deterioration (e.g. redundant storage/back-up files regularly updated, files maintained on limited-access servers/domains).
4. Physical records and archived materials will be maintained at FES, 1465 West 200 North, Logan, UT 84321.
5. Materials will be maintained indefinitely, unless an SOP, DWR policy, or other pertinent regulation/guidance allows for materials to be disposed of/destroyed. In instances where there is no such guidance available, FES Management (typically the FES Program Supervisor) will be consulted prior to disposal/destruction of any records or archived materials.
6. Materials may be accessed by any FES employee, however, FES Management (typically the FES Program Supervisor) must be consulted prior to removal or transfer of original documents or archived materials (other than copies) to non-FES personnel or to a location other than the FES.

F. QUALITY CONTROL
- N/A

G. INTERPRETATION
N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

General 200

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Personal Protective Equipment**	
SOP #: 201	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods to ensure proper personal protective equipment (PPE) is used. All personnel handling hazardous substances and/or conducting laboratory work at FES use PPE appropriate for such activities so as to protect them from health hazards associated with exposure to these materials and conditions.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate PPE when handling hazardous materials. To determine the appropriate personal protective equipment to be used for safely handling chemicals or other hazardous materials or to safely carry out laboratory duties, personnel should review pertinent SDSs, labeling (e.g., labels on chemical containers, decals on equipment indicating hazards or proper use), SOPs or user manuals for any tools, machinery or instruments and/or consult with the Fish Pathologist/Station Director, Fish Health Specialist and federal and State safety authorities.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Eye and facial protection such as safety glasses and face shields
2. Protective clothing such as lab coats, coveralls and aprons
3. Protective footwear such as close-toed shoes and steel-toed boots
4. Hand and dermal protection such as latex/nitrile gloves and barrier creams
5. Respiratory protective gear such as dust masks and respirators
6. Hearing protection such as ear plugs
7. Eyewash stations and/or emergency showers
8. Safety restraining devices

E. PROCEDURES

1. Personnel using tools, machinery and instruments must be trained in the use of such equipment and use the equipment in accordance with the pertinent SOP(s) and/or user manual(s), including use of the specified PPE.
2. Personnel using any solvents, chemicals, reagents or other hazardous materials must do so in accordance with the pertinent SDSs, including use of the specified PPE.
3. Personnel must complete training applicable to the activities in which they are engaged, including safety-related training involving descriptions of the use of PPE.

F. QUALITY CONTROL

- Ensure all PPE is in working order and is within expiry, and all chemicals are labeled and handled appropriately.

ORIGINAL

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. Safety Data Sheets (SDS)
2. Pertinent SOPs regarding particular reagents used

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **General Use and Maintenance of Equipment**	
SOP #: 202	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to ensure that the Fisheries Experiment Station (FES) equipment used for the collection or generation of fish pathology or related data/information is correctly used and maintained. It is the responsibility of the FES management (typically the laboratory supervisor), in conjunction with FES personnel, to ensure that all equipment is appropriately calibrated, cleaned, maintained in good working order and available for use as needed.

B. DEFINITIONS

1. FES management: Typically the laboratory manager or designee.

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. Prior to and during use of equipment:
   - All equipment should be inspected prior to and cleaned after each use to ensure consistent, high quality performance.
   - All equipment is to be used in accordance with the pertinent SOP(s) and user manual(s), including calibration/certification and documentation of such maintenance activities in the pertinent equipment use log.
2. Calibration:
   - All equipment requiring calibration (including all microscopes, balances, pipettes, thermometers, meters, incubators, refrigerators, freezers, autoclaves, hoods, spectrophotometers and other instruments), whether by a licensed subcontractor or FES management, should be calibrated against traceable certified equipment (e.g., National Institute of Standards and Technology, NIST) or a new, or recently certified, unit that can be traceable to a NIST standard as a reference.
   - FES equipment should be calibrated based on a timeline recommended by the manufacturer and whenever there is concern regarding the functioning or accuracy.
   - See Equipment Calibration/Maintenance Recommendations (Appendix A) for full descriptions of calibration intervals and parameters to be checked.
3. Damaged or malfunctioning equipment or outdated calibration/certification:
   - In the event of equipment failure or malfunction, FES management is to be notified immediately. Depending on the nature of the failure or malfunction, either the FES management or a licensed subcontractor will be responsible for completing the necessary repairs and/or recalibration.

ORIGINAL

- Notification/documentation should include a complete description of the problem, how the problem was discovered, the date equipment was removed from service (if applicable), the remedial action taken, the date of repair/recalibration/recertification, the date the equipment was placed back into service and a statement as to whether it was found to be in or out of calibration/standardization when removed from service.

4. Documentation:
    - Original documents/certificates of equipment inspections, calibrations, maintenance or other equipment related materials provided by outside vendors are to be filed with the equipment log.
    - Routine equipment testing, calibration and standardization procedures and/or service or repairs performed as a result of equipment failure or malfunction should be documented.
    - Records of calibration and maintenance documents are kept for each instrument.
    - Equipment logs are maintained for each piece of equipment in a designated, accessible location within the FES (e.g., the FES office). When appropriate, equipment maintenance and temperature information is posted on its surface (hoods, balances, incubators, etc.).
    - See any and all SOPs related to the use of a specific piece of equipment for full documentation on equipment calibration and maintenance.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. Any SOP(s) related to the use of a specific piece of equipment.
2. AFS-FHS (American Fisheries Society-Fish Health Section) FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See Equipment Calibration/Maintenance Recommendations (Appendix A) below.

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Appendix A

Equipment	External Calibration Interval	Internal Check Interval	Parameters to Check	Instruments Required
Autoclaves		Daily	Temperature & pressure sustained during operation	Pressure gauge, safety valve, temperature gauge, indicator strips
Balances	3 years	Weekly	Linearity, zero point, accuracy, level	Calibration reference weights
Biosafety Cabinets/Hoods	1 year	Weekly	Air flow, UV bulbs	Anemometer, vacuum meter
Centrifuges		During operation	Balance, speed, temperature, timer	Manufacturer provided
Eletrophoresis Units		Daily/ as used	Structural integrity, voltage	Volt meter (power unit)
ELISA Readers	1 year	Monthly	Lamp stability, optics, filters	Calibration plate
Freezers		Daily	Visual, thermal stability	Calibrated thermometer
Incubators		Daily	Temperature	Calibrated thermometer
Pipettes	3 years	Daily/ as used	Dirt and damage, volume delivery and accuracy	70% ethanol. If damaged, send for repair, graduated tips, gravimetric method.
Microscopes	3 years (cleaned)	Daily/ as used	Alignment, bulbs	Manufacturer procedure
pH Meters		Daily/ as used	Electrode drift or reduced response	Check against at least two buffer solutions.
Thermocyclers	If needed	Annually	Block temperature	Probe or check with sample replicate matrix
Thermometers (digital)	1 year	6 months	Check at point in working range against reference thermometer	Certified reference thermometer
Thermometers (liquid in glass)	10 years	6 months	Check at point in working range against reference thermometer	Certified reference thermometer
Timers		2 years	Accuracy	
Water Baths		Daily/ as used	Temperature and correlation with controls	Calibrated thermometer
Water Purification		Weekly/ as used	Conductivity and meter battery	Manufacturer provided

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Fisheries Experiment Station Fish Health Lab Cleaning Policy**	
SOP #: 203	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to ensure the laboratories and offices are clean and well maintained. A contracted janitorial service will cover the overall cleaning of the Fisheries Experiment Station (FES; vacuuming, mopping floors, restrooms, trash removal, dusting, cleaning sinks etc.). The janitorial service does not handle biohazard containers.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1] and proper ventilation when handling cleaning supplies such as alcohol, bleach, and benzalkonium chloride (Hyamine).

D. EQUIPMENT AND MATERIALS REQUIRED

1. Mop/bucket
2. Broom/dustpan
3. Autoclave bags (of various sizes)
4. Autoclave
5. 70% isopropyl alcohol
6. 10% bleach solution (made fresh)
7. DNA Away (commercial decontaminator)
8. Paper towels
9. Plastic tubs for bleach baths
10. Large disinfecting trough
11. Benzalkonium chloride (Hyamine)
12. Chlorhexidine

E. PROCEDURES

1. Disinfecting field gear:
    - Coolers returning from the field are decontaminated by submerging in the benzalkonium chloride (Hyamine) bath (1200 ppm) in the large disinfection trough located in the West lab. Coolers should remain submerged for at least 30 minutes, after which they are to be scrubbed and rinsed with fresh water before propping up to dry.
    - Reusable ice packs are disinfected in the same manner as coolers, described above.
    - Chairs and tables used during field inspection trips get a quick dip into the benzalkonium chloride (Hyamine) bath and are then scrubbed and immediately rinsed with fresh water. If these items are left in the benzalkonium chloride (Hyamine) bath, they will collect water.

- Waders/boots returning from off-site must be disinfected by a dip in the benzalkonium chloride (Hyamine) bath and then be rinsed with fresh water before hanging up to dry.

2. Disinfecting necropsy equipment:
    - Dissecting tools can be placed in a bleach bath for no longer than a few hours, as the bleach will corrode these instruments. Alternatively, the tools can be placed in a bath of chlorhexidine solution. The tools then must be scrubbed with laboratory soap, rinsed with fresh water and allowed to air dry completely. After drying, the tools can be packaged in autoclave pouches and autoclaved for 15 minutes at 121°C.
    - Cutting boards and other plastic supplies used during a necropsy must be placed in a bleach bath for at least 30 minutes. All equipment then must be scrubbed, rinsed and allowed to air dry
    - All other equipment that cannot be placed in a bleach bath or autoclaved must get sprayed and wiped down with either a bleach solution or alcohol preparation.
3. Biohazard bags:
    - Bags are gathered and autoclaved routinely. It is important to immediately autoclave biohazard bags when finished processing for particular assays (i.e., head biopsies, DNA extractions, etc.) even if the bags are not full so as the contaminated waste is not accumulating within the laboratory. When autoclaving is not possible, store biohazard bags in freezer.
    - Disinfect by autoclaving for 30 minutes at 121°C.
    - Once bags are cool enough to handle, remove from autoclave and place in dumpster in parking lot.
    - Biohazard bags are handled by FES employees only.
4. Spills:
    - Acid, caustic, solvent and formaldehyde spills are treated with the spill kits located in the main laboratory.
    - Immediately sprinkle the spill with the appropriate containment/neutralizing substance in the kit. Read the label for the time necessary to neutralize material before moving to a trash container.
    - Infectious materials/liquid spills are sprayed with 70% isopropyl alcohol and/or bleach.
        - Wear gloves and move material to biohazard bag.
        - All paper products, gloves etc. are put in the biohazard bag.

ORIGINAL

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Glassware and Plasticware Cleaning**	
SOP #: 204	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods of proper cleaning of all laboratory glassware.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Laboratory dish soap
2. Dish-washing brush or sponge
3. Bleach bath
4. Tap water
5. Distilled water
6. Aluminum foil

E. PROCEDURES

1. Glass bottles, small flasks and cylinders:
   - Rinse dirty glassware with tap water and place in bleach bath for at least 10 minutes.
   - Rinse glassware after removing from bleach bath and squirt with lab soap.
   - Use brush or sponge to gently hand wash both the inside and outside of each glass item (including any corresponding plastic caps).
   - Rinse glassware with tap water until all soap residue is removed.
   - As a final rinse to reduce water spots, rinse the glassware briefly with dH_2O.
   - Place glassware in dish drying rack next to sink or on the wall drying rack.
   - If needed, after glassware is completely dry, place cap on bottles or double line with aluminum foil, cover with autoclave tape and autoclave.
2. Plastic ware:
   - Disinfect plasticware (slide holders, PCR racks, tube racks, etc.) in 10% bleach bath and wash by hand.
   - Place in dish drying rack next to sink.
3. Contaminated glassware:
   - Autoclave to disinfect (i.e. glassware used for whirling disease).
   - Place in bleach bath for at least 10 minutes.
   - Use brush or sponge to gently hand wash both the inside and outside of each glass item (including any corresponding plastic caps).
   - Rinse glassware with tap water until all soap residue is removed.
   - As a final rinse to reduce water spots, rinse the glassware briefly with dH_2O.

ORIGINAL

- Place glassware in dish drying rack next to sink, or on the wall drying rack.
- If needed, after glassware is completely dry, place cap on bottles or double line with aluminum foil, cover with autoclave tape and autoclave.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Handling and Disposal of Sharps**	
SOP #: 205	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to properly handle and dispose of sharps used in the FES fish health lab.

B. DEFINITIONS

1. Sharps at the FES fish health lab include but are not limited to razor blades, scalpel blades, microtome blades, needles, dissecting scissors, forceps and microscope slides.

C. SAFETY PRECAUTIONS

- All sharps should be handled with caution and require the use of proper personal protective equipment[1].
- Safety glasses may be worn when risk of shattering will occur.

D. EQUIPMENT AND MATERIAL REQUIRED

1. Scalpel blades
2. 10% Bleach solution in discard container
3. Razor blades
4. Autoclave
5. Needles
6. Forceps
7. Microtome blades
8. Microscope slides
9. Safety glasses
10. Dissecting scissors
11. First aid kit

E. PROCEDURES

1. Any area where sharps are used should have a disposal or transport container for used sharps.
2. It is the responsibility of each to use proper care and handling of these items and to dispose of them properly. Be aware of the immediate environment and take steps to control the location of the sharp(s) to avoid injury.
3. All disposable sharps should be discarded in a sharps container specifically designed for that purpose.
4. Do not try to retrieve anything disposed of into the sharps container or overfill the container as per manufacturer instructions.
5. When disposing of a sharps container, make sure the lid is shut tightly and secure with autoclave tape. Autoclave sharps at 121°C for 15min and dispose of entire container in the dumpster when finished.

ORIGINAL

6. Non-disposable sharps should be thoroughly cleaned and disinfected in bleach or chlorhexidine. Instruments should then be autoclaved at 121°C for 15min.

F. QUALITY CONTROL

- Ensure each autoclave cycle is successful by using heat and pressure sensitive autoclave tape or instrument packaging.

G. INTERPRETATION

Do not dispose of sharps with other types of laboratory waste. Do not handle sharps in such a way that they are likely to cause injury. Sharps must be disposed of in an appropriate sharps container.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Handling of Hazardous Materials and Chemical Inventory**	
SOP #: 206	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to ensure that hazardous materials (e.g., chemicals, biological materials) in use at FES are acquired, stored and disposed of properly.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Review all appropriate SDS(s) when working with chemicals and reagents[1].
- Ensure proper disposal of all materials[2].
- Use personal protective equipment appropriately[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. General:
   - It is the responsibility of all FES personnel to ensure a safe working environment and that hazardous materials are acquired, stored and disposed of properly. Personnel must complete training applicable to the activities in which they are engaged, including safe handling of hazardous materials.
   - Refer to GEN 010 Position Descriptions, Curriculum Vitae/Resumes and Training Records for further information regarding required training, timelines for completion/renewal, etc.
   - All chemical handling is to be conducted in accordance with the pertinent SDSs and policies/regulations of the FES, NRDWR, State of Utah and/or federal safety authorities.
   - Concerns or questions regarding hazardous material acquisition, storage and disposal should be directed to FES management (typically the FES Program Supervisor).
2. Acquisition of hazardous materials:
   - FES management (typically the FES Program Supervisor) must be consulted prior to the purchase, transfer or use of any hazardous materials not on the existing inventory for the FES.
   - Before a hazardous material is used, information on proper handling, storage and disposal will be made available to those who have potential exposure. A SDS will be made available for all chemicals in use or on the current inventory.
   - No hazardous material container is to be accepted without adequate identifying label information. The label should include, at a minimum, the substance name, appropriate hazard warnings and identification of the manufacturer or distributor.

ORIGINAL

3. Inventory:
    - The chemical inventory for the FES, including any materials synthesized in the FES, is included in the Chemical Hygiene Plan.
    - Air, water, fish tissue and similar samples obtained by FES personnel staff will not be considered hazardous for inventory purposes, but will be appropriately contained, labeled (e.g., contents, sample identification number, etc.), stored and disposed of, based on the nature of the sample.
4. Storage:
    - Both the storage and working amounts of hazardous materials will be kept to a minimum.
    - All hazardous material containers must have a legible and firmly attached label.
    - All hazardous materials will be assigned an expiration date. The expiration date will generally be provided by the manufacturer or distributor. In cases where such an expiration date is not provided, hazardous materials will be assigned an expiration date of 1 year post-receipt in the laboratory.
        - Expiration dates will be added to container labels, if not indicated by the manufacturer or distributor. Additionally, the date of receipt in the lab will also be labeled on containers.
    - The hazardous material inventory will be assessed periodically and unneeded hazardous materials will be disposed of through the proper channels.
    - Acids/bases and flammable/combustible materials will be stored in appropriate containers and storage cabinets designed for such purposes. Flammable/combustible materials will not be stored in refrigerators/freezers unless the unit is rated for storage of such chemicals.
    - Compressed gas cylinders must be secured at all times bench straps, chains or other appropriate fasteners to secure the cylinders to a bench, wall or other immovable structure.
    - Incompatible chemicals must be segregated by class.
5. Disposal:
    - All hazardous waste will be disposed of through appropriate channels.
        - Chemical wastes will be disposed of in accordance with recommendations of the receiving authority (e.g., municipal landfill, academic Environmental Health and Safety program).
        - Disposal of biological waste is not directly addressed by this SOP and must be handled following the procedures described in SOP# 209, Disposal of Hazardous Waste.
        - Broken glassware (not containing hazardous waste or glass that is considered a puncture hazard) will be placed in rigid cardboard box, sealed with tape and disposed of in the same manner as FES household/municipal waste.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

- N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. Any SDS(s) related to a specific chemical (see online database or hard copy in office)
2. SOP#209, Disposal of Hazardous Wastes
3. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/**A**

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Shipping of Infectious Agents**	
SOP #: 207	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods used to pack and ship materials (including those that are potentially infectious) via FedEx or UPS.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution and proper personal protective equipment[1] when handling potentially infectious materials.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Cardboard box
2. Styrofoam box
3. Assorted Ziploc bags
4. Cotton balls
5. Paper towels
6. Tape
7. Ice packs
8. Dry ice
9. FedEx waybill

E. PROCEDURES

1. General packaging requirements:
    - For liquid samples, you must include four layers of packaging:
        - Use watertight containers for liquid specimens that have been sealed securely and then tape for an additional seal. If you place multiple fragile primary receptacles in a single secondary receptacle, they must be individually wrapped.
        - Place absorbent material (cotton balls, paper towels, etc.) between the primary and secondary receptacles, using enough material to absorb the entire contents of all primary receptacles. If samples are not liquid, no absorbent material is added but items are secondarily packaged.
        - Place primary package in secondary packaging consisting of a watertight sealed plastic bag, plastic canister or screw-cap can.
        - Final packaging consists of rigid outer packaging such as fiberboard or corrugated cardboard. Styrofoam alone may not be used.
2. Shipping on ice:
    - Items are packed as noted above and then placed in a leak-proof Styrofoam cooler in a cardboard box.

ORIGINAL

        - Commercial ice packs are inserted above and below the items in order to establish even cooling.
        - Conventional ice may be used if sealed in a plastic bag to prohibit leaking.
        - Final packaging consists of rigid outer packaging such as fiberboard or corrugated cardboard. Styrofoam alone may not be used. Add or write "On Ice" label to package.

3. Shipping on dry ice:
    - Items are packed as noted above and then placed in a collapsible Styrofoam cooler. The collapsible cooler allows for dry ice to degas as it thaws without causing contents to rupture.
        - Dry ice is broken and inserted above and below the items for even cooling. Take care not to "burn" skin or the tissues/articles packaged with dry ice.
        - Final packaging consists of rigid outer packaging such as fiberboard or corrugated cardboard. Styrofoam alone may not be used. Dry ice label UN1845 and weight must be added in order to ship items on dry ice. This must also be declared on the FedEx waybill.

F. QUALITY CONTROL

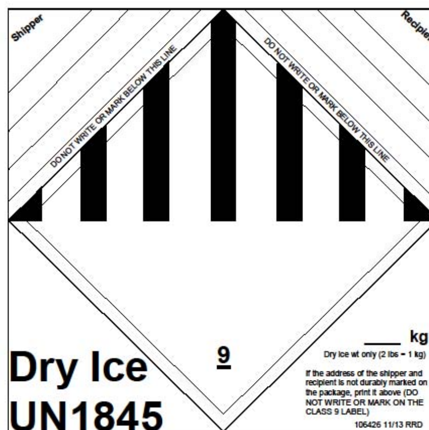
- Make sure the FedEx waybill is completely filled out. Tracking numbers included on waybill may be used to track shipments.

G. INTERPRETATION

FedEx pickup can be scheduled using the phone number detailed on the waybill. Keep the customer copy of the waybill for tracking and billing purposes.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. See following label:



ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Disposal of Infectious Materials**	
SOP #: 208	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the proper methods to disinfect and disposal of infectious materials.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Always wear appropriate personal protective equipment[1] when handling hazardous and infectious waste.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Biohazard bag
2. Autoclave indicator tape
3. Autoclave
4. 70% isopropyl alcohol
5. Sodium thiosulfate
6. Bleach

200 ppm is 3.8 mL bleach/liter waste

Volume waste	Bleach
5 L	19 mL
10 L	38 mL
15 L	57 mL
20 L	76 mL
25 L	95 mL
30 L	114 mL

Neutralization 1.5 g sodium thiosulfate per liter of bleached waste.

E. PROCEDURES

1. Biohazard bags and containers:
   - Biohazard containers are placed at work stations where fish or samples are processed. Bench top biohazard containers may be used when processing samples. Larger biohazard waste items may be placed in the large biohazard waste container situated in the center of the lab[2, 3].
   - Disposable items (towels, lab paper, Whirl Pak bags, tips, tubes, etc.) that have been in contact with specimens or blood are placed in biohazard bags.
   - Biohazard bags are taped closed and autoclaved at 121°C for 15-20 minutes.
   - Disinfected bags are then placed in trash containers.

ORIGINAL

- Large pieces of fish tissue and/or fish carcasses are not autoclaved on site at FES. Place fish carcasses in plastic garbage bag and place in freezer until they can be taken for incineration or disposal at the landfill.

2. Instruments (knives, forceps, scalpels etc.)[4]:
    - Carefully remove scalpel blades and discard in Sharps container.
    - Forceps, scissor, scalpel blade handles and knives etc. are to be placed in a bleach bath for at least 10 minutes (however, make sure to remove and rinse dissecting tools from bleach bath within 6 hours as bleach destroys metal).
    - After tools are rinsed and scrubbed with lab soap, they can be placed in dish drying rack until completely dry.
    - Place tools appropriately in autoclave packs (2 scalpel blades per pack, 2 pairs of forceps per pack, one pair of scissors per pack) and seal.
    - Autoclave for 15 minutes.
    - Knives are to be rinsed and scrubbed with lab soap and allowed to dry. After thorough cleaning, large knives do not need to be autoclaved.
3. Sharps containers[4]:
    - All glass microscope slides that have been used must be placed in Sharps containers.
    - All used needles must be placed in Sharps containers.
    - All used scalpel blades must be placed in Sharps containers.
    - Once Sharps containers are full they are to be securely closed and covered with autoclave tape and then autoclaved for 20 minutes before placing in municipal trash. Note: Sharps containers should never be in use for longer than 90 days, even if not completely full.
4. Disposables/equipment used in sample processing:
    - Virology plates/tubes are discarded in the biohazard bag in the virology room to be autoclaved.
    - Bacteriology plates/tubes and ELISA plates are discarded in the biohazard bag in the bacteriology room to be autoclaved.
    - Whirling disease:
        - Water used in straining and rinsing fish heads or wedges for whirling disease is collected in buckets which is disinfected with 200 ppm bleach for 30 minutes.
        - Bleach water and waste is discarded down the sink drain.
    - Any equipment used in sample processing must be placed in bleach bath for 30 minutes, then can be rinsed, scrubbed with lab soap and autoclaved (if glassware).

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
3. SOP# 209, Disposal of Hazardous Wastes
4. SOP# 205, Handling and Disposal of Sharps

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Disposal of Hazardous Wastes**	
SOP #: 209	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the proper methods for disposal of hazardous wastes.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Always wear appropriate personal protective equipment when handling hazardous waste[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Waste carboys for formalin and other hazardous waste collection
2. Fume hood
3. Necessary personal protective equipment

E. PROCEDURES

1. Collect all formalin waste in the safety carboy and work under fume hood[2].
2. Waste acids are stored in glass bottles in the acid cabinet.
3. Waste xylene is stored in the flammables cabinet.
4. Consult Fish Health Inspector and/or Fish Health Pathologist when wastes are ready to be disposed of.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Histology Preparation, Sectioning and Staining**	
SOP #: 210	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operation procedure (SOP) details the process of collecting and preparing samples for histological examination, including sectioning the samples and staining the slides.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Gloves and other personal protective equipment (lab coat, safety goggles, etc.) must be worn when staining slides and handling organic compounds such as xylene[1].
- Use caution when handling sharp microtome blades[2].
- Stain slides under an operating fume hood to limit the inhalation of harmful chemicals[3].
- Tissues are typically fixed in formalin, thus use caution when handling samples and formalin fixative.
- Dispose of hazardous wastes through appropriate channels[4].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Histology cassettes and lids
2. 10% neutral buffered formalin
3. Scalpel, microtome and razor blades
4. Charged and frosted microscope slides
5. Slide and staining racks
6. TissueTek™ staining containers
7. Xylene
8. Ethanol
9. Hematoxylin
10. Eosin
11. Bluing
12. Slide warmer
13. Permount
14. Coverslips
15. Paring knife
16. Paintbrush
17. Kimwipes
18. Paper towels
19. Paraffin
20. Filters

E. PROCEDURES

1. Tissue trimming and preservation:

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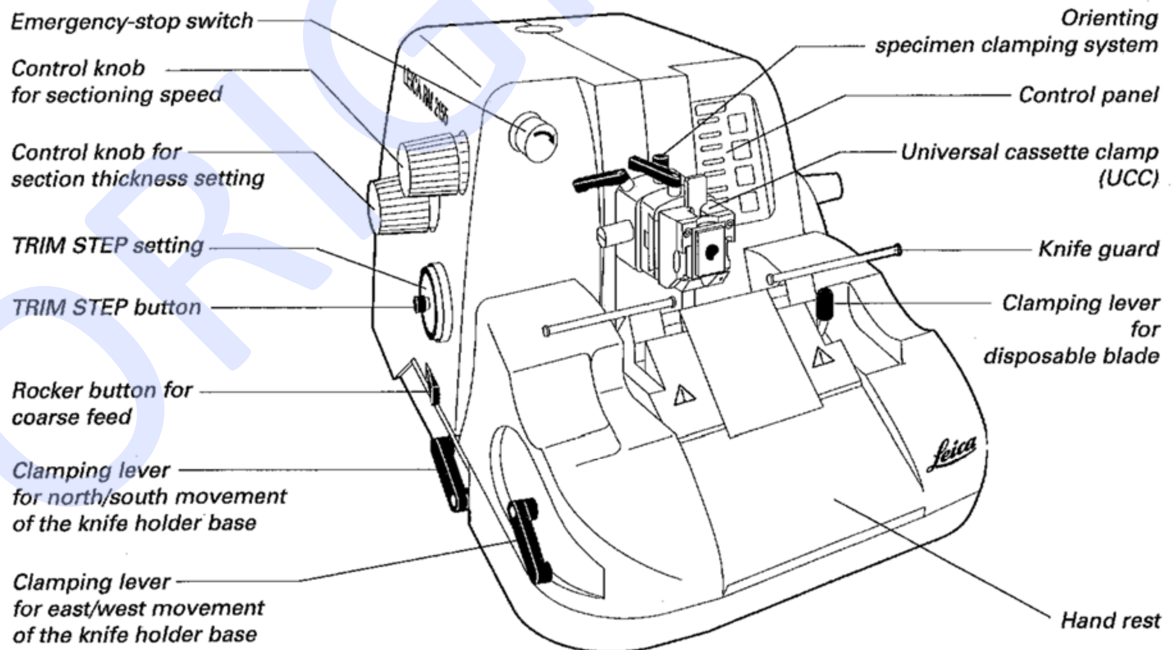
- Select desired tissue and trim with razor blade and scalpel to no more than 5 mm thick.
- Label tissue cassettes with case number and slide number using histo-marker or pencil.
- Place trimmed tissue inside cassette and snap lid shut.
  - Placement of tissue in cassette is important as it will be embedded as-is.
  - If smaller tissue fragments are present, a blue cassette sponge can be inserted to ensure no tissue is lost during sample processing.
- Place entire cassette in a specimen container of 10% neutral buffered formalin.
  - More than one cassette can be placed in each container.
  - Make sure formalin covers entire cassette.
- Submit entire container to histology laboratory at Utah Veterinary Diagnostic Lab (UVDL) using the accession form found online at http://www.usu.edu/uvdl/files/uploads/General_submission_form.pdf and drop off sample for processing (paraffin embedding).

2. Trimming and sectioning slides:
   - After paraffin embedded samples are retrieved from the UVDL after processing, prepare for trimming and sectioning slides.
   - Label all slides with case number, slide number and date.
   - Turn on slide warmer, water reservoir and cold or ice block.
   - Trim any excess paraffin off cassette with paring knife and place cassette in cassette clamp on microtome.
     - The clearance angle of the microtome blade should be set to 5°.



   - Face the slide at 20 µm until tissue plane is exposed.
   - Remove cassette from clamp and place on cold or ice block until ready to section.
     - When ready to section, set interval to 4 µm section until desired tissue is visible in wax ribbon.
   - Using a paintbrush, place tissue/wax ribbon in water reservoir so that it floats on the surface of the water.

ORIGINAL

        - Select section desired and tease it away from other sections and mount it on charged slide using paintbrush.
    - Blot any water from slide on a paper towel and allow to dry upright or directly on slide warmer.
        - Slides may be left at 56°C overnight if desired.
3. Hematoxylin and eosin staining:
    - Once slides are completely dry, slides are ready for deparaffinization, rehydration and staining.
    - Prepare all reagents in clean TissueTek™ containers to about 250 mL and place slides in staining rack.
        - Any precipitate formed in stains must be filtered out.
    - Prepare all reagents and set TissueTek™ containers up in hood according to the below schedule. Set timer and blot staining rack on paper towel if needed between stain changes.
        - Xylene or xylene substitute 10 minutes
        - Xylene or xylene substitute 10 minutes
        - 100% alcohol 1 minute
        - 100% alcohol 1 minute
        - 95% alcohol 1 minute
        - 95% alcohol 1 minute
        - Running tap water 10 minutes
        - Hematoxylin 10 minutes (may vary with tissue thickness)
        - Tap water 4 dips
        - Acid alcohol 3-10 dips
        - Tap water 4 dips
        - Bluing 5 dips
        - Running tap water 20 minutes
        - Eosin 2 minutes (may vary with tissue thickness)
        - 95% alcohol 2 minutes
        - 95% alcohol 2 minutes
        - 100% alcohol 3 minutes
        - 100% alcohol 3 minutes
        - Xylene or xylene substitute 2 minutes
        - Xylene or xylene substitute 2 minutes
    - Slides are now ready to coverslip by applying one drop of Permount to the slide (still wet with xylene) and coverslipping.
    - Clean microtome with xylene to remove wax buildup. Failure to clean the microtome thoroughly may result in inaccurate sectioning.
    - After use, cassettes containing tissue sections should be sealed with hot paraffin and can then be stored indefinitely.

F. QUALITY CONTROL

- Reagents should be made and used fresh or precipitate filtered out.

G. INTERPRETATION

ORIGINAL

Hematoxylin is deep blue to purple in color which intensely stains nucleic acids. Eosin is a bright pink stain which infiltrates proteins nonspecifically. Consult the fish health pathologist for interpretation of slides.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
4. SOP# 209, Disposal of Hazardous Wastes

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah

Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Preparation of Recirculation System Tanks for Challenge Trials**	
SOP #: 211	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: M. Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: General	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to detail the preparation of recirculation system tanks for a disease challenge trial.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (i.e., lab coat, gloves, safety goggles, etc.)[1].
- Bleach: Avoid contact with skin and eyes. Wear suitable gloves. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. If swallowed, seek medical advice immediately[2].
- Handle all pathogens with care and as if they are pathogenic to humans.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Bleach
2. Sodium thiosulfate
3. Bio-filter seed culture

E. PROCEDURES

1. System Disinfection:
    - Fill tanks and sump with water. Place covers and stand pipes inside the tanks so they are submerged and will be disinfected when bleach is added. Turn systems on so recirculation can occur.
    - Add 618 mL of bleach to each separate recirculating system. This should approximate a 200 ppm solution with a total water volume of 50 gallons per system.
    - Let the systems run several hours and clean miscellaneous parts with a brush or scour pad. Also clean the walls of the tanks and any surface that may have been contaminated during previous trial.
    - Remove pre-filter mats from the top of the sump and place in one of the tanks. Let the systems run overnight.
    - The next day remove pre-filter mats and place into a 5 gallon bucket for cleaning. Clean and squeeze them out well. If they are still serviceable, place back in a tank for subsequent neutralization, if not, discard and replace.
    - To save on the amount of sodium thiosulfate needed, reduce the water level using the inner standpipe. Keep the systems running with adequate water in the sump.
    - Weigh out crystal sodium thiosulfate (169 g/system; =0.74 mg/l per 1 ppm chlorine) and dispense into the tanks and let run for several hours to overnight.
    - Place the pump used previously in the sump of each tank and begin draining the system. At the same time place the fresh water line in one of the tanks and begin rinsing

out the headbox, tanks and sump so any residual chemicals or debris is flushed to the sump and removed.
- Give the tanks, covers and standpipes a thorough rinse and also lift the lid of the sump and rinse the filter media (Styrofoam peanuts).
- If the systems are to be left dry and shut down, turn off the fresh water, turn off the recycle system pump and let the pump inserted in the sump drain.
- If the systems are to be used again, remove the pump and let the system equalize with no standpipes in place until the sump adequately fills.

2. Seeding the Biofilter:
   - Add 10 mL of 6% ammonium chloride solution to each system. If a fertile system is already running, transfer aged water to use as a seed source or add recommended amount of commercially available bacterial seed culture.
   - After tanks have been running for 24 hour test for ammonia using the Hach kit. Record results and add another dose of ammonium chloride to tanks. This will provide the seed culture with nutrients needed for bacterial growth.
   - At 5 days post inoculation, re-test ammonia levels. If ammonia levels are below 2 ppm add another dose of ammonium chloride.
   - One week post inoculation, test for ammonia again and add another dose of ammonium chloride. Begin testing for nitrite concentrations.
   - Ten days post initial inoculation test nitrite using Hach kit. Add a half dose of ammonium chloride (5 mL).
   - At 15 days post inoculation re-test nitrite. Add a half dose of ammonium chloride (5 mL). This should imitate the presence of fish in the system. Once water quality reaches acceptable levels (Section G), fish can be added or water exchanges can begin with continued addition of ammonium chloride.

F. QUALITY CONTROL

- Water quality should be monitored throughout any trial. This will ensure that a healthy baseline will be achieved for all fish regardless of treatments.

G. INTERPRETATION

Suggested water quality criteria for optimum health of salmonid fishes (Piper et al. 1982).

Ammonia (NH_3)	0.0125ppm
Chlorine	0.03ppm
Nitrite	0.1 to 0.2ppm
Dissolved Oxygen	>5ppm
CO_2	0-10ppm
Temperature	42-60°F

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
3. Bleach Safety Data Sheet
4. Piper Et.al. Hatchery Operation, In: Fish Hatchery Management. 1982 pp. 60-130

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

Field Sampling 300

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Packing an Inspection Cooler**	
SOP #: 301	Date Initiated: 3/20/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/20/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/20/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods for packing an inspection cooler for hatchery and wild site inspections.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- MS-222 should be handled with extreme caution. Inhalation hazard[1].
- Use caution when handling sharp dissecting tools[2].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Net
2. Sharpies
3. Scalpel blades and handles
4. Forceps
5. Scissors
6. Cutting boards
7. Knife for heads
8. MS-222
9. Racks
10. Tubes, 15, 50 mL
11. Gloves
12. Butcher paper
13. Tape
14. Paper towel rolls
15. Ziplocks
16. Large garbage/biohazard bags
17. 70% alcohol
18. Bleach
19. Whirlpaks
20. Formalin specimen cups
21. Disinfection containers
22. Cide wipes
23. Slides
24. Craft sticks
25. Liquinox
26. Table
27. Chairs
28. Instrument containers

ORIGINAL

E. PROCEDURES

1. Pack all items listed in the supplies checklist (attached) orderly into a clean cooler.
   - More supplies may be needed for certain inspections sites.
   - Initial and date checklist, tape checklist to top of cooler.
2. When returning to station after cooler use, disinfect used materials and dispose of spent consumables.
   - Dispose of used sharps and replace needed cooler materials.
3. Disinfect cooler in hyamine bath, rise and allow to dry before re-packing.

F. QUALITY CONTROL

- Check off each item on supplies list as it is packed. This will ensure enough materials are on hand during each inspection.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
2. SOP# 205, Handling and Disposal of Sharps
3. See attached "Supplies Checklist".

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached supplies checklist.

ORIGINAL

Supplies Checklist

tape to cooler

Location: ______________________ Case: ______________

General Items for Inspections – Large Cooler

- ☐ Collection Form
- ☐ Dissection Supplies/ Instruments
  - ☐ Forceps – 6 minimum
  - ☐ Scalpel handles – 4 minimum
  - ☐ Scalpel blades – multiple
  - ☐ Scissors – 1 minimum
  - ☐ Craft sticks
    - ☐ Small ☐ Large
  - ☐ Used instrument container
  - ☐ Disinfection container – 2
  - ☐ Sharps container (50ml tube)
- ☐ Disposable supplies
  - ☐ Butcher paper/Large Bags
  - ☐ Masking tape
- ☐ Bags
  - ☐ Autoclave/ Biohazard - 4
  - ☐ Gallon Ziploc – 6
  - ☐ Quart Ziploc – 6
- ☐ Disinfection and cleaning supplies
  - ☐ Bleach – Nalgene Bottle
  - ☐ 'Cide wipes

- ☐ Sharpies, pens and pencil in a pouch
- ☐ MS-222
- ☐ Cutting boards - 3
- ☐ Large knife - fish head removal
  - ☐ Knife sharpener (if available)
- ☐ 70% alcohol - Nalgene Bottle
- ☐ 70% alcohol – 2 Spray Bottles
- ☐ Plastic 10% formalin containers - 4
- ☐ Virology rack
- ☐ Paper towels – 2 rolls minimum
- ☐ Aprons – 3 disposable
- ☐ Gloves: ☐ Medium ☐ Large
- ☐ Trash/Lawn bags - 4
- ☐ Whirl packs – 60
- ☐ Soap – Liqui-Nox or dish soap
- ☐ Hand Wipes

NOT IN COOLER

- **☐ Sample Cooler(s)**
  - **☐ BKD tubes and rack (if applicable)**
  - **☐ Labeled whirl packs for heads**
  - **☐ Test tubes for virology**
- **☐ Tubes for ovarian fluids (if applicable)**
- **☐ Ice packs**
- **☐ Additional Supplies**
  - **☐ Table**
  - **☐ Chairs**
  - **☐ Waders**
  - **☐ Disinfection tub/Scrub brush**
- **☐ 5 Gallon Bucket**
- **☐ Small net (☐ in Sample Cooler)**
- **☐ Electro-shocker**
- **☐ Nets for electro-fishing**

Comments: ____

Prepared by: ______________________ Date: ______________

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: General Necropsy and Sample Collection for Inspections**	
SOP #: 302	Date Initiated: 3/20/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/20/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/20/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods used to collect tissue and/or other biological samples from fish species in accordance with the Utah's Fish Health Certification Policies.

B. DEFINITIONS

Lot and Inspection Definitions

Note: Refer to Appendix B in this manual or Table 2.1 in the most recent version of the Bluebook (http://afs-fhs.org/bluebook/bluebook-index.php) for detailed information on what species of fish are susceptible to each pathogen and the conditions under which it is most readily detected.

1. **Lot of Non-Broodstock Fish:**
   A group of non-brood fish of the same species and age group that have continuously shared a common water source throughout their life history. A representative sample of all strains and rearing units containing this lot shall be included.
2. **Lot of Broodstock Fish:**
   A group of sexually mature fish of the same species that share a common water source. The sample must be representative of all age groups (e.g. three-, four-, and five-year-old brood fish) and strains present at the facility.
3. **Lot Inspection:**
   The collection and examination of a statistically valid number of the appropriate samples from a specific lot of a susceptible species for any pathogen listed in this manual. Moribund fish will be included when present. Unless otherwise stated in the policies and/or regulations of the jurisdictions involved, sampling for the required pathogens will be performed at the 5% APPL with a 95% confidence level. See Table 2 in this manual or table 2.3 in the most recent version of the Bluebook for further explanation of the number of samples required.
   - Exception
     In broodstock lots where there is access to ovarian fluid, sampling for the required viral pathogens will be performed at the 5% APPL with a 95% confidence level in both kidney/spleen tissues and ovarian (coelomic) fluid. Kidney/spleen and ovarian fluid samples must come from different individuals.
     - *Example:* in a broodstock population consisting of 2500 individuals, kidney/spleen samples will be collected from 60 fish (males and/or females) and ovarian fluid will be collected from an additional 60 females for a lot inspection requiring IHNV testing.
   - Exception
     A lot of anadromous salmon regularly monitored for *Renibacterium salmoninarum* through ELISA or QPCR techniques may be considered positive for this pathogen

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without additional testing. Results of the monitoring must be provided to the jurisdiction involved when requested.

4. **Facility Inspection:**
   Inspection of each and every susceptible lot of fish held on the facility for any of the bacterial, viral and parasitic pathogens listed in this manual is required.
   - Exception
     For *Myxobolus cerebralis* and *Renibacterium salmoninarium*, only one lot of the most susceptible species on each water source at the facility needs to be inspected. It is essential that the lot chosen has had sufficient exposure to create a detectable infection.
   - Inspection Frequency
     Most regulating jurisdictions require that a history of annual inspection be submitted with the inspection report prior to permitting the importation, stocking, and/or transfer of aquatic animals. It is therefore, recommended that a program of annual facility inspections be encouraged for any facility participating in intrastate, interstate, and/or international commerce of their animals.
5. **Sample Number:**
   Unless otherwise dictated by the receiving jurisdiction, the number of fish to be collected from each lot must be in accordance with a plan that provides 95% confidence that at least one infected fish will be collected if the minimum assumed pathogen prevalence level (APPL) of infection equals or exceeds 5%. Examples of the number of fish to sample for various population sizes are listed in Table 2. Table 2 also includes examples of the number of fish to sample if a 2% or 10% APPL is required by the requesting authority. If the population size is estimated to be between two grouping levels, the sample is taken from the next higher population class.

Table 2. Sample number based on an assumed pathogen prevalence level (APPL) in the population of 10%, 5%, or 2%.

Population Size	Number of Fish Required for Sample		
(number of fish)	10% APPL	5% APPL	2% APPL
50	20	35	50
100	23	45	75
250	25	50	110
500	26	55	130
2000	27	60	145
>100,000	30	60	150

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1].
- Take caution when handling sharp dissecting tools (scalpels and knives)[2].
- Use appropriate caution when working with MS-222[3].

D. EQUIPMENT AND MATERIAL REQUIRED

1. Completed Hatchery Inventory Request Form (Appendix A)
2. MS-222 or Aqui-S
3. Medium or large transport cooler depending on number of lots
4. 2 - 5 frozen ice packs depending on size of cooler

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5. Rack of 60 sterile BKD tubes and caps containing 100 of sterile 70% ethanol enclosed within a Ziploc bag
6. 15 mL centrifuge tubes containing 5 ml transport media for viral tissue samples enclosed within Ziploc bag (x12 per lot)
7. 50 mL centrifuge tubes for ovarian fluid samples in Ziploc bag (x12 per lot)
8. Empty 15 mL tube rack
9. Cutting board
10. Coolers and ice packs
11. Sterile dissection tools:
    - Forceps
    - Scalpel handles
    - Scalpel blades
    - Scissors
    - Craft sticks
12. 2 Plastic cups for disinfection and rinsing of dissection tools between lots
13. 2 Nalgene bottles each containing 200 mL 70% ethanol for disinfecting tools between lots
14. 2 spray bottles containing 70% ethanol
15. Plastic formalin container
16. Trash bags
17. Whirl packs
18. Soap-Liquinox
19. One gallon Ziploc bags for heads (1-2 required depending on size of heads)
20. Large knife for head removal
21. Multiple rolls of paper towels
22. Butcher paper
23. Sharps container
24. Masking tape
25. Used instrument container
26. Sharpie pen, ink pen, pencil, ruler
27. Small Clorox container (50-100 mL)
28. Cide wipes
29. Latex or nitrile examination gloves
30. Lab coat or apron

E. PROCEDURES

1. Facility Information:
    - Tour hatchery facility with manager or staff personnel.
    - Verify fish on site match inventory submitted.
    - Determine fish lots and numbers of fish to be sampled from each lot.
    - Unpack cooler and set up supplies and equipment.
        - *Note: Keep transport media in cooler and use only as needed.*
    - For multiple lots, transfer 70% ethanol into plastic disinfection cups. Add enough solution to submerge dissection tools while collecting samples.
    - Request disposal container for carcasses and biological waste.
    - Request disposal container for processing debris (paper towel, gloves, etc.).
2. Sample Selection:
    - All samples shall be processed as soon as possible after collection.

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- If the collected animals are not maintained alive before processing, samples shall be stored chilled (0 to 4°C) but not frozen and shall be processed as soon as possible after collection.
- All samples for virology must be inoculated onto cell cultures within 72 hours post-collection.
- Fish selected should be represent the lot of fish being inspected and shall include fish with lesions and moribund fish when present.

3. Size/Age Groups:
   - Environmental and species differences can markedly affect the growth rate of fish. In addition, some pathogens are most readily detected when fish are a certain size, whereas others are most readily detected when fish are a certain age.
   - For the purpose of fish health inspections, fish are assigned to one of four groups based on either size or age depending on the pathogen of interest.
   - Table 1 provides a general reference for these classifications. These classifications may not fit all species.

Table 1. Suggested categories for grouping for sample collection.

Designation	**Total length**	**or**	**Age**
Fry	< 4 cm		0–3 months of age
Fingerlings	4 – 6 cm		4-12 months age
Yearlings/Adults	> 6 cm		Non-brood fishes greater than 12 months of age
Broodstock	> 6 cm		Sexually mature fish greater than 12 months of age and used as broodstock

4. General Necropsy and Sample Collection:
   - Request hatchery personnel net fish samples and ensure they use minimal water in container.
   - Cover container to keep larger fish from escaping.
     - *Note: MS-222 or Aqui-S can be used to anesthetize fish.*
   - Process smaller fish first and work up to larger fish at hatcheries with multiple lots.
     - *Note: Change gloves between lots.*
   - Examine and note presence of gross external lesions. If lesions are collected for histological examination, it must be done in a manner that will not compromise the aseptic collection of samples for bacteriology and virology.
   - Collected fish are humanly euthanized immediately prior to sample collection.
     - *Note: If confirmation of M. cerebralis infection is to be done histologically, fish should not be killed by a blow to the head as this may compromise the integrity of skeletal elements.*

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- Fry are generally only examined for viruses. Fingerling, yearling and adult sized fish may be examined for bacteria, virus and/or parasites.
- The instruments used during sample collection are at a minimum cleaned between sample pools and disinfected between lots.
- Make an incision just ventral to the midline from the isthmus to just anterior to the vent.
- Cut an opening to facilitate access to visceral organs (Figure 1).
    - *Note: The body cavity is opened, being careful not to compromise the target sample tissues with contents from the intestinal tract. Exercise extreme caution so the visceral cavity is not contaminated with the scalpel, forceps, or fingers.*
- If it blocks access to the kidney, the swim bladder is moved.
- Collect required tissue based on fish species, age and size requirements in accordance with virology, bacteriology and parasitology protocols.

Figure 1: Trout necropsy.

5. Post inspection clean up:
    - Remove scalpel blade and place in sharps container.
    - Clean dissection tools with Clorox.
    - Bag and seal all trash generated by collection.
    - Recount samples taken and lot numbers.
    - Pack sample cooler taking care to store virology and bacteriology sample in upright position.
    - Record information on collection report and place in cooler.
    - Deliver cooler to processing lab.

F. QUALITY CONTROL

- Maintain appropriate aseptic technique so visceral cavity and samples are not contaminated with scalpel, forceps or fingers.

G. INTERPRETATION

Observe specimen internally and externally and make note of any clinical signs of an infectious agent.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 817, MS-222 (Tricaine Methanesulfonate)

ORIGINAL

4. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.
5. Colorado Parks and Wildlife. Aquatic Animal Health Laboratory Procedures Manual. 2016.

I. REVISION HISTORY

1. Original

J. APPENDICES

- 302.A Appendix: Inspection Collection Form
- 302.B Appendix: Pathogen And Sample Matrix

ORIGINAL

UTAH DIVISION OF WILDLIFE RESOURCES
FISH HEALTH INSPECTION REPORT

FES Case Number:
Fish Health Approval Number:

Name of Fish Source: Address or Location: UTM North UTM East	Name of Owner or Manager:	Inspection Date(s): January 0, 1900	Results:

FISH EXAMINED

Lot Number	Species[1]	Age/Length[2]	# in Lot	Obtained as Eggs (E) or Fish (F) from:

PATHOGENS INSPECTED FOR AND RESULTS[3]

Rs	VE	VH	VP	Mc	AT	A	B

Collector(s):

Type of Fish Examined:

Hatchery ☐ Wild ☐
Salmonid ☐ Non-Salmonid ☐

Type of Water Supply:

Enclosed ☐ Well ☐
Spring ☐
Free of Fish ☐ Stream ☐

Remarks:

- ☐ Unless otherwise noted, this inspection represents entire source sampling
- ☐ Ovarian fluids not available for IHNV testing
- ☐ Parental stocks tested and found free of IHNV
- ☐ A = EPC (15+22°C) and BF-2 (22°C) cell lines used for viral assay
- ☐ B =

Inspecting Biologist Signature

Concurring Signature

Certified Fish Health Inspector

AFS Certified Fish Health Inspector and Address:
Wade P. Cavender, M.S., Certified Fish Pathologist
Fisheries Experiment Station, 1465 West 200 North, Logan, UT 84321-0202
(435)752-1066

1 Use standard abbreviations (see species list and abbrev)
2 For hatchery fish, give age in months
3 See list of pathogen abbreviations; finding reported as number examined/results, where (-) is negative and (+) is positive

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Appendix 1. A matrix to assist in selecting fish, the appropriate tissue, screening and confirmation methods to use to detect the specific pathogens listed below.

ORGANISM	COMMON NAME OF DISEASE	KNOWN SUSCEPTIBLE SPECIES	TISSUE FOR SAMPLING	PRIMARY (SCREENING TECHNIQUE)	CONFIRMATORY TECHNIQUE	COMMENTS
Bacterial Pathogens						
Aeromonas salmonicida	Furunculosis	Any freshwater fish	Kidney	Bacterial culture of kidney on TSA or BHIA media	Fluorescent Antibody Test (FAT)	May be isolated from many species of fish, birds, and protozoan parasites
Yersinia ruckeri	Enteric Red Mouth (ERM)	Any freshwater fish	Kidney	Bacterial culture of kidney on TSA or BHIA media	FAT	May be isolated from many species of fish and birds
Edwardsiella ictaluri	Enteric Septicemia of Catfish (ESC)	Ictalurids	Kidney	Bacterial culture of kidney on TSA or BHIA media	FAT	
Renibacterium salmoninarum	Bacterial Kidney Disease (BKD)	Salmonids	Kidney, ovarian fluid	Direct fluorescent antibody test on kidney smear or ovarian fluids	Bacterial culture using SKDM-2 media for a total of 6 weeks *or* nested Polymerase Chain Reaction (PCR) technique	
Piscirickettsia salmonis		Salmonids, freshwater, and marine fish	Kidney/Spleen/Liver/Blood	Cell culture on CHSE for 28 days. Hold for an additional 14 days. Or tissue impression stained with Giemsa.	IFAT, Immuno-histochemistry or PCR	Use antibiotic-free media in cell cultures.
Viral Pathogens						
Infectious Hematopoietic Necrosis Virus	IHN	Salmonids	Whole fry, viscera, or kidney/spleen - depending on size, ovarian fluid	Cell culture on EPC cells for 14 days at 15°C. Followed by a 14-day blind pass.	Serum neutralization or nested PCR or IFAT	Target tissues should be kidney/spleen from larger fish and ovarian fluid from spawning broodstock.
Infectious Pancreatic Necrosis Virus	IPN	Wide variety of freshwater and saltwater fish and shellfish	Whole fry, viscera, or kidney/spleen - depending on size, ovarian fluid	Cell culture on CHSE-214 cells for 14 days at 15°C. Followed by a 14-day blind pass.	Serum neutralization or nested PCR or IFAT	Target tissues should be kidney/spleen from larger fish and ovarian fluid from spawning broodstock. May be isolated from many species of aquatic organisms
Infectious Salmon Anemia Virus	ISA	Salmonids and Atlantic herring	Whole fry, viscera, or kidney/spleen - depending on size; ovarian fluids	Cell culture on SHK-1 cells for 14 days at 15° C. Followed by a 14-	PCR or IFAT	In addition to sampling kidney spleen, when available sample ovarian fluid from spawning

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ORGANISM	COMMON NAME OF DISEASE	KNOWN SUSCEPTIBLE SPECIES	TISSUE FOR SAMPLING	PRIMARY (SCREENING TECHNIQUE)	CONFIRMATORY TECHNIQUE	COMMENTS
				day blind pass.		broodstock. Most mortality occurs in saltwater with fluctuating temperatures
Oncorhynchus masou Virus	OMV	Salmonids	Viscera, ovarian fluids	Cell culture on CHSE-214 cells for 14 days at 15° C. Followed by a 14-day blind pass.	PCR technique or send to reference lab for confirmation	Target tissues should be kidney/spleen from larger fish and ovarian fluid from spawning broodstock. Only known to occur in Japan
Viral Hemorrhagic Septicemia Virus	VHS	Salmonids, pike, turbot, herring, pilchard	Kidney/spleen	Cell culture on EPC cells for 14 days at 15° C. Followed by a 14-day blind pass.	PCR	In addition to sampling kidney spleen, when available sample ovarian fluid from spawning broodstock.
White Sturgeon Herpesvirus	WSHV	White sturgeon, possibly shortnose sturgeon	Kidney/spleen, ovarian fluids	Cell culture on WSS-2 cells for 14 days at 20° C. Followed by a 14-day blind pass.	Send to reference lab for confirmation	
Largemouth Bass Virus	LMBV	Centrarchids and ecocids	Kidney/spleen/swim bladder	Cell culture on FHM or BF-2 cells for 7 days at 25 to 30° C. Followed by a 7-day blind pass.	PCR	
Spring Viremia of Carp Virus	Infectious carp dropsy	Cyprinids, also brown trout, pike, shrimp and copepods	Kidney/spleen	Cell culture on EPC cells for 14 days at 20 to 25° C. Followed by a 14-day blind pass.	Serum neutralization or PCR	Most easily isolated in the spring during and for several weeks after epizootics.
Parasite Pathogens						
Myxobolus cerebralis	Whirling Disease	Salmonids	Cranial cartilage (entire head or wedge/core sample from larger fish	Pepsin-trypsin digest	Histological observation of spores/lesions consistent with infection in cranial cartilage or nested PCR	For a facility inspection only one lot of the most susceptible species on each water source need be inspected. When possible select fish that have been on that water supply, while at a susceptible age, for a minimum of 1800 degree-days C or for six (6) months.
Ceratomyxa shasta	Ceratomyxosis	Salmonids	Intestine (posterior)	Wet mounts of intestinal scraping	Detection of spores or PCR	When possible select fish 1) in earth ponds or ponds receiving untreated surface water, 2) that have been on that water supply

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ORGANISM	COMMON NAME OF DISEASE	KNOWN SUSCEPTIBLE SPECIES	TISSUE FOR SAMPLING	PRIMARY (SCREENING TECHNIQUE)	CONFIRMATORY TECHNIQUE	COMMENTS
						for a minimum of six (6) months and 3) that are moribund or lethargic.
Tetracapsula bryosalmonae	Proliferative Kidney Disease (PKD)	Salmonids	Kidney	Smears of kidney stained with Leishman-Giemsa or Lectin	Histology	When possible: 1) select fish from earth ponds or raceways receiving untreated surface water, 2) sample moribund fish and 3) conduct sampling during summer or early fall months.
Bothriocephalus acheilognathi	Asian Tapeworm	Cyprinids, silurids, poeciliids, percids, centrarchids, gobiids, cyprinodontids	Intestine (anterior one third)	Visualization of cestode with pyramidal scolex in the semi-contracted state	Positive identification by use of a key	Late summer and fall sampling optimal for detection.

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Field Collection of Bacteriology Samples**	
SOP #: 303	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to ensure proper methods are utilized for the collection of bacteriological samples in the field in order to support accurate diagnostic results.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1].
- Take caution when handling sharp dissecting tools (scalpels and knives)[2].
- Use appropriate caution when working with MS-222[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Scalpel and blades
2. Scissors
3. Forceps
4. Bacteriology loops
5. Appropriate agar plates
6. Parafilm
7. Gram stain slides
8. 12 well slides
9. Frosted glass slides
10. Slide holder
11. Paper towels
12. Butcher paper
13. Cutting board
14. Alcohol spray bottle
15. Gloves
16. Sharpie marker
17. MS-222
18. Cooler and ice packs

E. PROCEDURES

1. Examine fish externally and note surface abnormalities (e.g., frayed fins, cloudy eyes, ulcers, skin discolorations, parasites and tumors).
2. Sample any lesions or areas of interest with a scalpel, loop or other appropriate method to obtain tissue.
3. Streak the specimen on Tryptic Soy Agar and TYES Agar bacteriological plates, label and seal the plates.

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4. Prepare tissue imprints of affected areas on frosted glass slides for Diff-Quik and Gram staining. Remove a piece of tissue, blot on a clean paper towel to remove most of the blood and lightly touch the cut surface of the tissue to a clean frosted glass or gram stain slide. Several imprints from the same piece of tissue can be made on one slide. Air dry the preparation and heat fix. Place slides in a slide holder for transport.
5. Prepare tissue imprints of affected areas (as described above) on 12 well glass slides and fix slides for BKD DFAT and FL-43 staining.
6. Spray fish with alcohol to disinfect and wipe the exterior surface clean.
7. Open the visceral cavity through the abdominal wall. Avoid cutting into the intestinal tract. Note any abnormalities. Expose the kidney by removing the swim bladder and note any kidney abnormalities.
8. Insert a sterile loop into the kidney and streak the specimen on Tryptic Soy Agar and TYES Agar bacteriological plates, label and seal the plates.
9. For spleen samples, aseptically remove the spleen and place on a clean frosted slide.
10. Insert a sterile loop into the spleen and streak the specimen on Tryptic Soy Agar and TYES Agar bacteriological plates, label and seal the plates.
11. Prepare tissue imprints of affected areas on frosted glass slides for Diff-Quik, on gram stain slides for gram staining and on 12 well slides for FAT and FL-43 staining as described above.
12. If taking samples for histology, use only very freshly euthanized fish (1-10 minutes). Remove small sections of tissue and place in Davidson's solution fixation for further analysis.

F. QUALITY CONTROL

- Ensure aseptic technique is used during all sample collections.

G. INTERPRETATION

Samples are to be analyzed further in laboratory setting.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 817, MS-222 (Tricaine Methanesulfonate)
4. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Field Collection of *Renibacterium salmoninarum* DFAT Samples**	
SOP #: 304	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This Standard Operating Procedure (SOP) describes the collection, alcohol fixing and transport of kidney samples for *Renibacterium salmoninarum* direct fluorescent antibody test (DFAT[1]). This method will diminish chances of cross contamination and decrease the chances of false positive findings.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[2].
- Take caution when handling sharp dissecting tools (scalpels and knives)[3].

D. EQUIPMENT AND MATERIAL REQUIRED

1. All materials described in General Necropsy Techniques[4]
2. 12x75 mm, 5 mL culture tubes
3. 100% Molecular grade ethanol
4. Small cooler with ice packs

E. PROCEDURES

1. Examine and necropsy fish as described in General Necropsy.
2. Aseptically collect small (approximately 5mm) section of kidney (target anterior section if possible) and place in a pre-filled 5 mL culture tube containing 500 µL molecular grade ethanol.
3. Make sure forceps are clean (dip in 70% isopropyl alcohol) and repeat for each fish in desired lot(s).
4. Samples from each lot of fish are separately bagged, labeled and placed in a cooler.

F. QUALITY CONTROL

- Samples must be collected aseptically. Tools, equipment and gloves are changed between lots of fish.

G. INTERPRETATION

Samples are to be processed and analyzed further in laboratory setting.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 414, *Renibacterium salmoninarum* Direct Fluorescent Antibody Test
2. SOP# 201, Personal Protective Equipment
3. SOP# 205, Handling and Disposal of Sharps
4. SOP# 302, General Necropsy Techniques

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5. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Field Collection of *Flavobacterium psychrophilum* Fluorescent Antibody Test (FAT) Samples**	
SOP #: 305	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the techniques used to obtain a field sample that may provide corroboration for a presumptive diagnosis of Bacterial Cold Water Disease (BCWD) that is faster than standard bacterial culture.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure safety and caution when using sharp tools[1].
- Ethanol is flammable; avoid open and direct flame[2].

D. EQUIPMENT AND MATERIALS REQUIRED

1. All materials as described in General Necropsy[3]
2. All materials as described in Tissue Collection of Field Samples for Bacteriology[4]
3. Sterile swabs
4. 5 mL plastic tubes
5. Phosphate buffered saline (PBS)
6. 100% ethanol

E. PROCEDURES

1. Examine fish. Observe for clinical signs typical of BCWD: moderate anemia, bloody ascites, swollen spleen and/or kidney, gill lesions, muscle ulcerations and fin erosions.
2. Using aseptic technique and the procedures described in General Necropsy. Remove a piece of the spleen using forceps.
   - Dab and smear the spleen tissue onto a clean well of a labeled 12-well microscope slide. The result should be a light film of cells with minimum blood or other tissues.
   - This technique can be used on other tissues (e.g., kidney, gill, muscle ulceration, fin, etc.) if deemed necessary during gross exam. Sterile swabs or inoculating loops may be used to transfer sample to slide.
   - Allow the slide to air-dry before placing in a slide container and transporting back to the laboratory.
3. Alternatively, fish or tissues that have been collected aseptically and stored in phosphate buffered saline (PBS) or 100% ethanol may be transported whole back to the laboratory for processing.
   - Place samples in a chilled container for transport to the laboratory.
   - Samples should be stored at 4°C for processing within 24 hrs.
   - Samples should be placed at -80°C for long term storage.

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F. QUALITY CONTROL

- Use aseptic technique throughout tissue collection. Sterilize tools between tissue collection of different fish. Change tools and equipment between lots.

G. INTERPRETATION

Samples are to be analyzed further in laboratory setting utilizing the *F. psychrophilum* Fluorescent Antibody Test.[5]

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 205, Handling and Disposal of Sharps
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
3. SOP# 302, General Necropsy and Sample Collection for Inspections
4. SOP# 303, Tissue Collection of Field Samples for Bacteriology
5. SOP# 415, *Flavobacterium psychrophilum* Fluorescent Antibody Test.
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Field Collection of Samples for Virology**	
SOP #: 306	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods used to collect aseptic virology samples in order to support accurate diagnostic results.

B. DEFINITIONS

1. TM: Transport media
2. K/S: Kidney/spleen
3. WF: Whole fish
4. WV: Whole viscera
5. OF: Ovarian Fluid

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1] and ensure safety when working with sharp tools (scalpels and knives)[2].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Scissors
2. Scalpel and blade
3. Forceps
4. Small cooler with ice packs
5. Tube rack
6. Transfer media (TM)
7. Gentamycin (purchased pre-prepared)
8. 15 mL tubes
9. 50 mL conical tubes

E. PROCEDURES

1. Assemble the appropriate number of collection tubes for tissues to be sampled and place in west lab refrigerator.
    - Kidney/spleen samples: 15 mL tubes filled with 5 mL TM[3].
    - Ovarian fluid samples: 50 mL conical tubes filled with 100 µL gentamycin if samples will not be processed within 6 hours of collection.
    - For a routine collection of 60 fish, 12 collection tubes for tissue or OF are needed per lot when samples are collected in 5 fish pools.
    - Label tube caps with lot number.
2. Examine and necropsy fish as described in General Necropsy[4].
3. Collecting tissues for virology[5]:
    - Juvenile fish ≤ 7.0 cm in length:
        - Fish 2.5 cm or less – use whole fish after removing the yolk sac.
        - Fish 2.5 to 4.0 cm – cut off and discard heads and tails.

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        - Fish 4.0 to 7.0 cm – use viscera.
    - Juvenile fish > 7.0 cm:
        - Kidney and spleen.
    - Adult fish:
        - Females – kidney and spleen or OF from spawning fish.
        - Males – kidney and spleen or milt if organs are not available.
    - Collect virology tissues by placing 1-5 samples of kidney/spleen, whole viscera, or whole fish into 15 mL tubes.
        - These samples should be taken after BKD samples have been collected.
        - Sample volume range should be estimated between 0.5 – 1.5 g.
        - For diagnostic cases, tissue from individual fish may be collected to increase assay sensitivity.
    - Rinse necropsy tools in 70% isopropyl alcohol between each fish pool or between each individual fish if warranted.
        - Change tools between lots.
    - Collect OF by draining the eggs from 1-5 fish through a sieve into a receptacle and pouring off about 30 mL (a minimum of 2 mL should be collected from each fish) into a 50 mL tube.
        - Alternatively, collect samples directly from uro-genital opening with a pipettor.
        - Milt may be expressed into a clean paper cup and 2 mL transferred to a collection tube.
    - Keep all filled tubes in a cooler on ice while collecting remainder of samples and during transport.
4. Transport back to FES on ice and place at 4°C in virology lab.

F. QUALITY CONTROL

- Samples must be collected aseptically.
- Tools, equipment and gloves are changed between lots of fish.

G. INTERPRETATION

Samples are to be processed and analyzed further in a laboratory setting.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 834, Transfer Media (TM)
4. SOP# 302, General Necropsy and Sample Collection for Inspections
5. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Field Collection of *Tetracapsuloides bryosalmonae* Samples**	
SOP #: 307	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods to ensure proper field collection of samples to be tested for the presence of *Tetracapsuloides bryosalmonae* in support of accurate diagnostic results.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use protective gear[1] (gloves and eye protection) when working with MS-222[2].
- Exercise extra precaution when using sharp tools[3] (scalpels and knives).

D. EQUIPMENT AND MATERIALS REQUIRED

1. All materials as described in General Necropsy[4]
2. Clean microscope slides

E. PROCEDURES

1. Examine and necropsy fish as described in General Necropsy.
2. Collection of kidney and/or spleen tissue:
   - Aseptically collect a portion of the posterior kidney and/or spleen.
   - Make a tissue imprint using the sample by gently dabbing the tissue on the surface of a clean microscope slide. Take care to ensure limited tissue adheres to the slide as this will inhibit further analysis.
   - Repeat for all samples to be collected.
   - Allow slides to completely air dry before transporting back to laboratory for further analysis.

F. QUALITY CONTROL

- Take care to label all slides and samples and limit cross contamination between cases/lots. Use new and/or cleaned tools between sample collections.

G. INTERPRETATION

Samples are to be processed and analyzed further in a laboratory setting utilizing techniques associated with SOP 508[5].

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling Of Hazardous Materials And Chemical Inventory
3. SOP# 205, Handling And Disposal Of Sharps
4. SOP# 302, General Necropsy And Sample Collection For Inspections

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5. SOP# 508, *Tetracapsuloides bryosalmonae* Sample Processing And Identification
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Field Collection Of *Ceratonova shasta* Samples**	
SOP #: 308	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This Standard Operating Procedure (SOP) describes the procedure to ensure proper methods are utilized for the collection of *Ceratonova shasta* samples in the field in order to support accurate diagnostic results.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use protective gear[1] (gloves and eye protection) when working with MS-222[2].
- Ensure safety when handling sharp dissecting instruments[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. All materials as described in General Necropsy[4]

E. PROCEDURES

1. Examine fish and dissect as described in General Necropsy.
2. Collect all other samples prior to sampling the intestine for *Ceratonova shasta.*[5]
3. Remove the posterior 1/3 of the intestine and place the sample into a transport container such as a Whirl-pak or Petri dish.
4. Transport samples on ice and examine within 24 hours.

F. QUALITY CONTROL

- Ensure aseptic technique is used during all sample collections.
- Process only one case at a time to limit the risk of cross-contamination between lots.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
3. SOP# 205, Handling and Disposal Of Sharps
4. SOP# 302, General Necropsy and Sample Collection for Inspections
5. SOP# 509, *Ceratonova shasta* Sample Processing and Identification
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

ORIGINAL

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Field Collection of *Bothriocephalus acheilognathi* Samples**	
SOP #: 309	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods for the collection of fresh gastrointestinal tract tissue for later examination for the presence of adult Asian tapeworm (*Bothriocephalus acheilognathi*).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use protective gear[1] (gloves and eye protection) when working with MS-222[2].
- Ensure safety when using sharp tools (scalpels and knives)[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. All materials as described in General Necropsy[4]
2. Petri dishes
3. Phosphate Buffered Saline[5]

E. PROCEDURES

1. Examine and necropsy fish as described in General Necropsy.
2. Make an incision in the ventral wall of the fresh (or formalin fixed) specimen. Cut into the body cavity and to the anus without severing the intestine.
3. Cut the intestinal tract at the anus and at the esophagus or just posterior to the stomach and remove the intestine with forceps.

4. Line up section(s) of intestine on the top of the small side of a Petri dish (see below).

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F. QUALITY CONTROL

- Handle samples with gloves and ensure no cross contamination between samples and especially between cases/lots.

G. INTERPRETATION

See SOP# 507, *Bothriocephalus acheilognathi* Sample Processing and Identification.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
3. SOP# 205, Handling and Disposal of Sharps
4. SOP# 302, General Necropsy and Sample Collection for Inspections
5. SOP# 821, Phosphate Buffered Saline (PBS)
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Field Collection f *Myxobolus cerebralis* Samples**	
SOP #: 310	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Field Sampling	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods to ensure proper field collection of samples to be tested for the presence of *Myxobolus cerebralis* in support of accurate diagnostic results.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use protective gear[1] (gloves and eye protection) when working with MS-222[2].
- Exercise extra precaution when using sharp tools (scalpels and knives)[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. All materials as described in General Necropsy[4]
2. Scalpel blades and handles
3. Scissors
4. Large knives

E. PROCEDURES

1. Examine and necropsy fish as described in General Necropsy.
2. Collect entire fish head using scalpels, scissors or knives.
3. All head samples from the same case can be placed in one labeled plastic bag.
4. Larger fish can have a wedge removed as described in Section 2 of the Blue Book.
5. Samples transported to laboratory in chilled container (i.e., cooler with ice packs and/or ice).
   - Samples to be frozen at -20°C for long term storage when analyzing by PCR only (no samples to be processed using the pepsin-trypsin digestion (PTD) method should ever be frozen).
   - Samples to be placed at 4°C when sample processing will take place within 24 hours.

F. QUALITY CONTROL

- Collect samples from one case at a time and change tools, equipment and gloves between lots of fish.

G. INTERPRETATION

Samples are to be processed and analyzed further in laboratory setting.

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
3. SOP# 205, Handling and Disposal of Sharps
4. SOP# 302, General Necropsy and Sample Collection for Inspections
5. SOP# 501, Head Defleshing Procedure in Preparation for Pepsin-Trypsin Digestion (PTD)
6. SOP# 502, Pepsin-Trypsin Digestion (PTD) for the Recovery of Myxobolus cerebralis Spores
7. SOP# 505, Processing Head Samples in Preparation for DNA Extraction
8. SOP# 708, *Myxobolus cerebralis* Polymerase Chain Reaction; Single Round
9. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

Bacteriology 400

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Sampling and Handling Tissue for Streaking on Bacterial Agar Plates**	
SOP #: 401	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate sampling and handling methods for streaking tissues on bacterial agar plates. This SOP is intended to guide the collection and handling of samples for bacteriology.

B. DEFINITIONS

1. N/A

SAFETY PRECAUTIONS

- Use proper personal protective equipment when working with all bacterial cultures[1].
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures and dispose of properly[2, 3].
- Disinfect all surfaces before and after working with any bacterial cultures.
- Use care when working with sharps[4].

C. EQUIPMENT AND MATERIALS REQUIRED

1. All materials described in General Necropsy
2. All materials described in Tissue Collection of Field Samples for Bacteriology
3. Disposable bacteriology loops
4. Appropriate agar plates
5. Parafilm

D. PROCEDURES

1. Sample collection – general information:
   - Follow procedures for General Necropsy[5] and Field Collection of Bacterial Samples[6].
   - For the detection and identification of bacterial pathogens in populations of fish showing disease signs, ideal samples are multiple (five or more) moribund fish or those showing clinical signs typical of the disease outbreak. For the detection of subclinical infections in populations of asymptomatic fish, larger sample numbers may be necessary. Fish that are found dead at the time of sampling are not acceptable for bacteriological examination unless they are known to be very fresh. Contaminating bacteria can grow quickly in dead fish, particularly in warm water.
   - The selection of tissue samples for bacteriological assays varies depending on the pathogen suspected; the reader is referred to chapters on specific bacterial diseases. Organs most commonly tested include the spleen and kidneys and portions of any organ with visible lesions, although other samples such as the brain, blood plasma or ovarian fluid are used for the detection of certain bacterial infections.
   - Samples for attempted culture of bacteria must be taken aseptically. If samples are to be taken from internal organs, disinfection of the body surface is recommended before

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incisions are made to expose the organs. Sterile tools must be used for making incisions and removing samples for culture.
  - Samples intended for Gram stains or immunological tests also should be taken aseptically to prevent contamination with bacteria or antigens from extraneous sources.
  - If samples cannot be inoculated immediately onto appropriate culture media, they may be stored on ice for up to 24 hours. Samples for culture should not be frozen.
2. Streaking of sample on media plates:
   - Bacteriological samples are aseptically removed from fish, (i.e. kidney, spleen, brain, lesions, etc.) and inoculated onto media plates.
   - A sample is dabbed onto one small area, and then streaked with a sterile inoculating loop (Fig. 2).
   - The loop is passed through the first streak and spread further out into the media (Fig. 3).
   - A third pass is made with the loop for the final streak (Fig. 4).
   - Label, seal with parafilm and invert plates.
   - Store at appropriate temperature for each sample.
3. Subculturing isolates:
   - A sample colony from mixed culture or masked by overgrowth is streaked for isolation onto fresh media.
   - The edge of the colony with the newest growth is collected on a loop and inoculated onto media Fig. 1).

Fig. 1

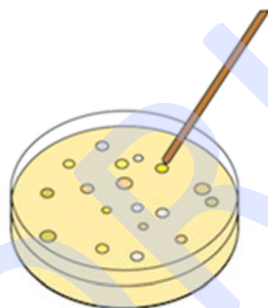

Fig. 2

Fig. 3

Fig. 4.

http://www.shmoop.com/prokaryotes/postulate-petri-dish.html

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E. QUALITY CONTROL

- Use aseptic technique to limit cross-contamination and work on samples from one case at time.

F. INTERPRETATION

Samples will be further analyzed in a laboratory setting.

G. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 209, Disposal of Hazardous Wastes

4. SOP# 205, Handling and Disposal of Sharps
5. SOP# 302, General Necropsy and Sample Collection for Inspections
6. SOP# 303, Field Collection of Bacteriology Samples

H. REVISION HISTORY

1. Original

I. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Staining of Tissue Smears and Imprints**	
SOP #: 402	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) details the process of staining blood smears and/or tissue imprints for the presence of pathogens.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1] (PPE) when handling methanol, slides and fresh tissues/fluids.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Methanol 100%
2. Deionized water
3. Giemsa stain prep (Sigma #GS500)
4. Slide dryer
5. Coplin jar
6. Diff-Quik solutions

E. PROCEDURES

1. Fix slides in 100% methanol or fixative for 20 minutes.
2. Air dry slides.
3. Geimsa
   - Submerge slides in Coplin jar to stain for 15-60 minutes depending on intensity desired.
   - Rinse in deionized water.
   - Air dry and read slides.
4. Diff-Quik
   - Stain with Diff-Quik solution II for 30 seconds.
   - Counterstain with Diff-Quik solution I for 30 seconds.
   - Rinse in tap water.
   - Air dry and read slides.

F. QUALITY CONTROL

- Make sure stain is within expiry and discard if precipitate has formed.

G. INTERPRETATION

Geimsa: Nuclei will stain purple and cytoplasm will stain varying shades of blue to light pink. Basophils will stain dark blue and eosinophils will stain orange.

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Diff-Quick: Nuclei will stain dark blue and cytoplasm will stain pale pink. Basophils will stain dark purple and eosinophils will stain red.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 812, Preparation of Giemsa Stain
3. SOP# 302, General Necropsy and Sample Collection for Inspections

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Anaerobic Culturing**	
SOP #: 403	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure for anaerobic culturing of tissue samples when a pathogen is presumed to be anaerobic (i.e., Botulism/*Clostridium botulinum* in rainbow trout).

B. DEFINITIONS

1. BSL-2: BioSafety Level 2 pathogens require restricted access to the laboratory when work is being conducted and all procedures are conducted in biosafety cabinets. No benchtop work is permitted**.**

C. SAFETY PRECAUTIONS

- *Clostridium* spp. are human and animal pathogens that can cause gastrointestinal disease. Appropriate biosafety precautions must be observed (BSL-2).
- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic[2].
- Use aseptic technique when working with live bacterial cultures.
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Brain heart infusion (BHI) agar
2. Sterile petri dishes
3. Anaerobic chamber
4. GasPak EZ anaerobic sachet or equivalent
5. Disposable inoculating loops

E. PROCEDURES

1. Media preparation:
   - Prepare BHI agar per manufacturer instructions[3].
2. Pre-inoculation:
   - For best results, pre-reduce agar plates before use by placing them in the anaerobic chamber with a GasPak EZ anaerobic sachet overnight.
   - GasPak EZ anaerobic sachet must be free from visible rips and tears before opening. GasPak must be placed in a sealed anaerobic chamber no more than 2 minutes after opening.
     - Anaerobic indicator must clearly indicate that anaerobiasis has been achieved or process must be repeated.
3. Inoculation:
   - Collect tissue from desired area of fish with a sterile, disposable inoculating loop.
     - Streak for isolation on BHI agar.
   - Label petri dish and place in anaerobic chamber with GasPakEZ.

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    - When chamber is opened and plates are inspected for growth, a new GasPakEZ sachet must be used.
  - Dispose of all spent cultures and media by autoclaving.

F. QUALITY CONTROL

- Anaerobic indicator included with GasPakEZ must signify anaerobiasis. See package insert for additional information.

G. INTERPRETATION

There are few anaerobic pathogens of concern for Utah salmonids. *Clostridium* spp. has been detected in rainbow trout however and if suspected, proper safety precautions should be taken (BSL-2). The pathogen produces opaque, cream colored shiny colonies on BHI agar. The fish pathologist should be notified upon confirmation of this organism.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 803, Brain Heart Infusion (BHI) Agar

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Gram Staining Procedure**	
SOP #: 404	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods for performing a Gram stain. A Gram stain is used to differentiate bacteria as Gram-positive or Gram-negative based on cell wall composition.

B. DEFINITIONS

1. N/A

SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures[2].
- Disinfect all surfaces before and after working with any bacterial cultures.

C. EQUIPMENT AND MATERIALS REQUIRED

1. 12-well slide with fixed Gram stain controls
2. Inoculating loop
3. Flame or heat source for fixing slide
4. Phosphate Buffered Saline (PBS)
5. Gram stain kit, including crystal violet, Gram's iodine, decolorizer and safranin
6. Tap water
7. Transfer pipette
8. Bunsen burner and flint starter
9. Immersion oil
10. Microscope

D. PROCEDURES

1. Prepare a bacterial smear from a pure culture:
   - Put a drop of PBS[3] on a clean glass slide.
   - Using a sterile loop, touch an isolated bacterial colony and mix it with the drop of PBS (light inoculum is best, excess bacteria will not stain properly).
   - Let air dry and heat fix. Do not overheat.
2. Place slide on a slide rack in the sink.
3. Flood the slide with crystal violet and leave on the slide for 60 seconds.
4. Wash off the crystal violet with running tap water.
5. Flood the slide with the iodine solution and leave on the slide for 60 seconds.
6. Wash off with running tap water.
7. Decolorize with decolorizer solution until the solvent flows colorless from the slide (approximate 5 to 10 seconds). Excessive decolorization should be avoided because it may

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result in a false gram negative reading. Too little decolorization can result in a false positive reading.

8. Rinse immediately with running tap water.
9. Flood slide with safranin and leave on slide for 60 seconds.
10. Wash with running tap water and allow to air dry.
11. Thoroughly rinse sink.
12. Microscopically examine the slide for bacterial organisms under the 100x objective with immersion oil. Observe numerous fields and describe the Gram reaction of any organisms seen.

F. QUALITY CONTROL

- Use commercially prepared Gram stain control slides.

G. INTERPRETATION

1. Gram negative organisms stain pink to red.
2. Gram positive organisms stain deep violet to blue.
3. When reporting findings, you should also include:
   - Numbers of bacteria (scant, moderate, numerous, etc.); and
   - Morphology of the cells present.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 821, Phosphate Buffered Saline (PBS)
4. American Fisheries Society-Fish Health Section (AFS-FHS) Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens (most recent edition). Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached diagram.

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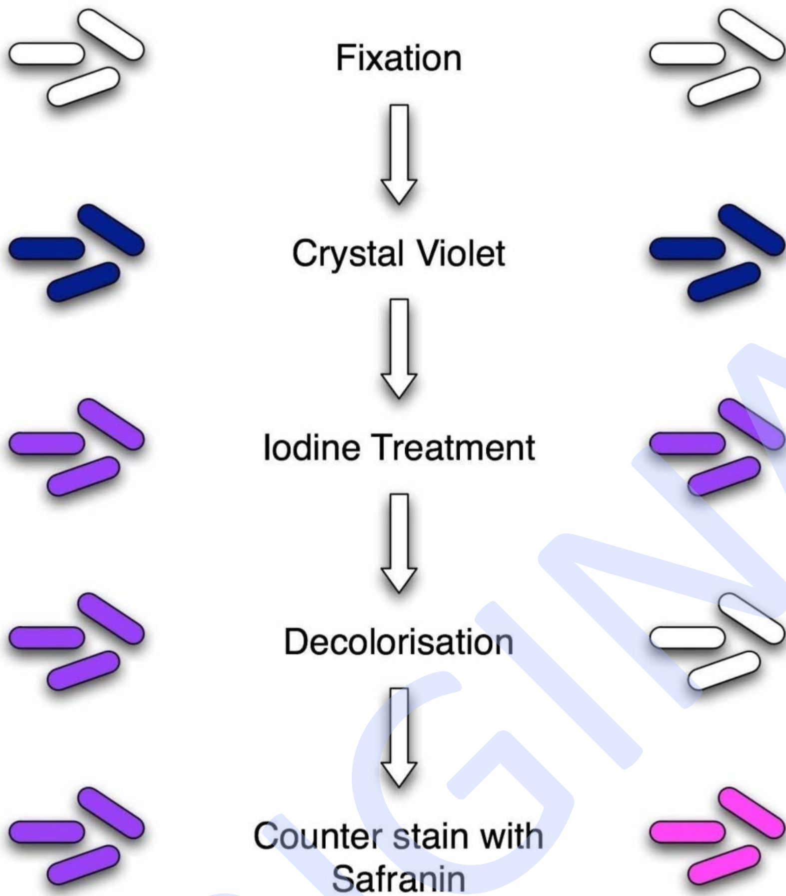

http://microbeonline.com/key-facts-about-gram-staining-techniques-that-you-might-not-know/

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Potassium Hydroxide (3%) Test for Gram Reaction**	
SOP #: 405	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods to perform a potassium hydroxide (KOH) test (also known as string test) to differentiate Gram negative from Gram positive bacteria.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure safety when handling reagents[1].
- KOH causes serious eye damage and severe skin burns.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sterile loop
2. Glass slide
3. 3% Potassium hydroxide solution
4. Bacterial cultures to be tested

E. PROCEDURES

1. Add a medium-heavy inoculum (visible amount) of a pure culture of bacteria grown on a solid medium to a drop of 3% potassium hydroxide (KOH) solution (3.0 g KOH per 100 mL distilled water) on a clean glass slide.
2. Stir for about 5 seconds to 1 minute, occasionally lifting the loop to look for thickening and "stringing" of the mixture.

F. QUALITY CONTROL

- Test known controls each time the test is performed (Gram positive: *Staphylococcus* spp.; Gram negative: *Flavobacterium psychrophilum*).
- False positives can occur with too heavy an inoculum or when using mucoid colonies.
- False negatives can occur when using too little inoculum or too much KOH.
- This test can be performed on older cultures successfully.
- Always double check results with traditional Gram stain procedure[2] if there is doubt about test results.

G. INTERPRETATION

Gram positive bacteria will not appear to change the viscosity of the KOH solution. Gram negative bacteria will cause the KOH solution to become stringy or viscous in appearance and consistency.

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 404, Gram Stain Procedure
3. American Fisheries Society-Fish Health Section (AFS-FHS) Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens (most recent edition). Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Bacterial Identification Using API 20 E**	
SOP #: 406	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: M. Hansen/C. Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) details the use of the API 20 E standardized identification system for classification of *Enterobacteriaceae* and other non-fastidious Gram-negative rods.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic[2].
- Use aseptic technique when working with live bacterial cultures.
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIALS REQUIRED

1. API 20 E strip
2. API incubation box with lid
3. API Suspension medium (5 mL)
4. API 20 E results sheet
5. API 0.85% NaCl (5 mL)
6. JAMES reagent
7. TDA reagent
8. VP 1 reagent
9. VP 2 reagent
10. Sterile mineral oil
11. McFarland standard
12. Oxidase test
13. Filter paper
14. 1 mL transfer pipette or 1 mL pipettor
15. Plastic loop
16. Plastic squeeze bottle with distilled water
17. Bacterial cultures to be tested

E. PROCEDURES

1. Strip preparation:
   - Set-up incubation tray and lid.
   - Label tray with case number, isolate number, date and identifying initials.
   - Dispense 5 mL of distilled water from plastic squeeze bottle into the incubation tray to provide humidity.
   - Remove a 20 E strip from packet and place in the incubation tray.

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2. Initial bacterial suspension:
   - Using a plastic loop, pick 1-4 well-isolated colonies with identical morphology previously sub-cultured from a single colony. Insert the loop into 5.0 mL sterile 0.85% NaCl and homogenize colonies to a turbidity of 0.5 McFarland standard.
3. Strip inoculation:
   - The 20 E strip contains 20 cupules and connecting tubes. The cupule is the upper portion of the tube.
   - With a sterile transfer pipette or pipettor inoculate the tube and cupule of CIT, VP and GEL with the bacterial suspension. Fill the tubes by placing the pipette tip against the side of the cupule while slowly adding the bacterial suspension.
   - Fill only the tubes of the remaining tests.
   - Overlay ADH, LDC, ODC, H2S and URE with mineral oil until a convex meniscus is formed.
4. Strip incubation:
   - Place the lid on the tray after inoculation and incubate at 15-22°C (depending on the selected culture) for 24 hours.
   - Incubation times may vary.

F. QUALITY CONTROL

- Make sure all reagents and test strips are within expiration dates.
- Non-fastidious Gram-negative rods can be inoculated onto API 20 NE strips (for non-enteric, catalase positive organisms) or API 20 E strips (for enteric, catalase negative organisms) for possible identification.
- Follow API 20 NE or API 20 E instructions for inoculation, interpretation of reactions and code identification charts. Refer to the package insert for more detailed information.
- When the API biochemical tests are unreactive, it may be necessary to use other types of media for identification purposes.
- Refer to package insert for more detailed information.

G. INTERPRETATION

1. Reading API 20 E strip:
   - Depending on growth patterns of the bacteria, read the strip after 24 -48 hours and record results on the API worksheet. Note: If the number of positive tests (before adding reagents) is less than 3, reincubate the strip for another 24 hours without adding the reagents.
   - Record all spontaneous reactions for tests ONPG, ADH, LDC, ODC, URE, GEL, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY and ARA on the result sheet.
2. TDA test:
   - Add 1 drop of TDA reagent to the TDA cupule. A reddish brown color after 5 minutes is a positive reaction and yellow is a negative reaction.
3. IND test:
   - Note: This test should be run last as the production of some gaseous products can interfere with interpretation of other test results. Add 1 drop of JAMES reagent to the IND cupule. A pink color is a positive reaction.
4. VP test:
   - Add 1 drop of VP1 and 1 drop of VP2 reagents to the VP cupule. A pink color reaction after 10 minutes is considered a positive reaction.

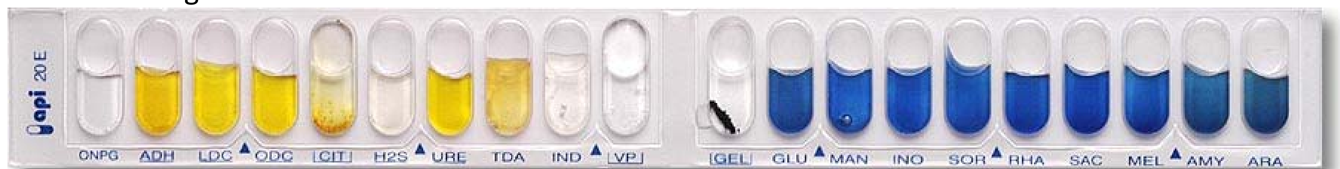
ORIGINAL

5. Assimilation tests:
   - Oxidase test[3] results should be included on the report sheet in order to accurately determine the final profile.
6. Report sheet and identification:
   - Mark positive and negative reactions on the report sheet at the appropriate time frame (see note above).
   - Log in to the Biomerieux website at https://apiweb.biomerieux.com/. User name: cchrisheck, password: fishlab. Pick the option for API 20 E. Record results including oxidase test. Choose confirm on the bottom right. Print out results page.

Tests	Negative	Positive
ONPG	colorless	yellow
ADH	yellow	red/orange
LDC	yellow	red/orange
ODC	yellow	red/orange
CIT	Pale green/yellow	Blue green/blue
H^2S	Colorless/greyish	Black deposit/thin line
URE	yellow	red/orange
TDA	TDA/immediate	
	yellow	reddish/brown
IND	James/immediate	
	Colorless/pale green/yellow	pink
VP	VP1 +VP2/ 10 min	
	colorless	pink/red
GEL	No diffusion	Diffusion of pigment
GLU	Blue/blue green	Yellow/greyish yellow
MAN	Blue/blue green	yellow
INO	Blue/blue green	yellow
SOR	Blue/blue green	yellow
RHA	Blue/blue green	yellow
SAC	Blue/blue green	yellow
MEL	Blue/blue green	yellow
AMY	Blue/blue green	yellow
ARA	Blue/blue green	yellow
OX	see oxidase package insert	

ORIGINAL

A. Negative tests



B. Positive tests

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 408, Oxidase Test
4. SOP# 404, Gram Staining Procedure
5. SOP# 407, Bacterial Identification Using API 20NE
6. SOP# 410, Presumptive Bacterial Identification Procedures
7. SOP# 411, Flowchart for the Presumptive Identification of Bacterial Fish Pathogens
8. Package insert for API 20 E
9. Biomerieux website: https://apiweb.biomerieux.com/

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Bacterial Identification Using API 20 NE**	
SOP #: 407	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: M. Hansen/C. Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) details the use of the API 20 NE standardized system for the identification of non-fastidious, non-enteric Gram-negative rods (*Pseudomonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Moraxella* spp., *Vibrio* spp., *Aeromonas* spp., etc.).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic[2].
- Use aseptic technique when working with live bacterial cultures.

D. EQUIPMENT AND MATERIAL REQUIRED

1. API 20 NE strip
2. API incubation boxes
3. API AUX medium
4. API 20 NE results sheet
5. 0.85% saline 5 mL tube
6. JAMES reagent
7. NIT 1 reagent
8. NIT 2 reagent
9. Zn reagent
10. Sterile mineral oil
11. McFarland standard
12. Oxidase test
13. Filter paper
14. 1 mL transfer pipette or 1 mL pipettor
15. Plastic loop
16. Plastic squeeze bottle with distilled water
17. Bacterial cultures to be tested

E. PROCEDURES

1. Strip preparation:
   - Remove one incubation tray and lid from the box.
   - Label tray with case number, isolate number, date and identifying initials.
   - Dispense 5.0 mL of distilled water from plastic squeeze bottle into the incubation tray to provide humidity.
   - Remove a 20 NE strip from packet and place it in the incubation tray.
2. Initial bacterial suspension:

ORIGINAL

- Using a plastic loop, pick 1-4 well-isolated colonies with identical morphology previously sub-cultured from a single colony. Insert the loop into a tube containing 2.0 mL of sterile 0.85% saline solution and homogenize colonies to a turbidity of 0.5 McFarland standard.

3. Strip inoculation:
   - The NE strip contains 20 cupules and connecting tubes. The cupule is the upper portion of the tube.
   - With a sterile transfer pipette or pipettor inoculate the tube portion of the first 8 tests (NO3, TRP, GLU, ADH, URE, ESC, GEL, and PNPG) with the saline/bacterial suspension. Fill the tubes by placing the pipette tip against the side of the cupule while slowly adding the bacterial suspension.
   - Open an ampule of AUX medium as directed and add 200 µL of the bacterial suspension to the Aux ampule. Mix well with a sterile transfer pipette or 1.0 mL pipettor taking care to not create bubbles.
   - Fill both the tubes and cupules of the next 12 tests of GLU through PAC so that the solution in the cupule is flat or slightly convex and not concave.
   - Overlay the GLU, ADH and URE tubes with mineral oil until a convex meniscus is formed.
4. Strip incubation:
   - Place the lid on the tray after inoculation and incubate at 15-22°C (depending on the selected culture) for 24 hours.
   - Incubation times may vary based on the organism and some results are not valid for 48 or more hours.

F. QUALITY CONTROL

- Make sure all reagents and test strips are within expiration dates.
- Non-fastidious gram-negative rods can be inoculated onto API 20 NE strips (for non-enteric, catalase positive organisms) or API 20 E strips (for enteric, catalase negative organisms) for possible identification.
- Follow API 20 NE or API 20 E instructions for inoculation, interpretation of reactions and code identification charts.
- Refer to the package insert for more detailed information.
- When the API biochemical tests are unreactive, it may be necessary to use other types of media for identification purposes.
- NOTE: Some non-enteric gram negative rods are oxidase negative (*S. maltophilia*, *Acinetobacter* etc). These microorganisms may also be identified with API 20NE but their selection must be based on other bacteriological or clinical criteria.
- NOTE: Fastidious organisms that have demanding nutritional requirements and/or require other handling precautions (i.e. *Brucella* and *Francisella*) are not included in the API 20 NE database. Alternative procedures must be used to exclude them or confirm their presence.

G. INTERPRETATION

- Reading API 20 NE strips:
  - Depending on growth patterns of the bacteria, read the strip after 24 to 48 hours (or other time frame as is required by the organism) and record results on an API worksheet.

ORIGINAL

- Record all spontaneous reactions for tests GLU, ADH, URE, ESC, GEL and PNPG on the result sheet.
- NO3 test:
  - Add 1 drop of NIT 1and 1 drop of NIT 2 reagents to the NO_3 cupule. A red color after 5 minutes is a positive reaction.
  - If there is a negative reaction (no color), add 2-3mg zinc dust to the cupule. If after 5 minutes the cupule remains colorless this is considered to be a positive reaction. If the cupule turns pink-red, the reaction is negative.
- TRP test:
  - Add 1 drop of JAMES reagent to the TRP cupule. An immediate pink color reaction is considered to be a positive reaction.
- Assimilation tests:
  - Examine cupules of tests GLU–PAC for bacterial growth. Opaqueness in the cupule indicates bacterial growth and is recorded as a positive reaction.
  - Note: If identification is not valid prior to 48 hours incubation time, aspirate the NIT 1, NIT 2 and James reagents and overlay the NO_3 and TRP tubes with mineral oil.
- Report sheet and identification:
  - Mark positive and negative reactions on the report sheet at the appropriate time frame(s).
  - Log-in to the Biomerieux website at https://apiweb.biomerieux.com/. User name: cchrisheck, password: fishlab. Choose the option for the API 20 NE and record results including the oxidase test[3]. Choose the confirm button at the bottom right and print out the results page.
  - See also AFS Blue Book section 3.A2 for profiles specific to fish pathogens *Yersinia ruckeri* and *Aeromonas salmonicida* which may differ from results generated by the API website.

Tests	Negative	Positive
NO_3	NIT 1 + NIT 2 5 min	
	colorless	pink-red
	Zn 5 min	
	pink	Colorless
TRP	JAMES immediate	
	colorless / pale green / yellow	Pink
GLU	blue to green	Yellow
ADH	yellow	orange / pink / red
URE	yellow	orange / pink / red
ESC	yellow	grey / brown / black
GEL	no pigment diffusion	diffusion of black pigment
PNPG	colorless	Yellow
GLU	transparent	Opaque
ARA	transparent	Opaque
MNE	transparent	Opaque
MAN	transparent	Opaque
NAG	transparent	Opaque

ORIGINAL

MAL	transparent	Opaque
GNT	transparent	Opaque
CAP	transparent	Opaque
ADI	transparent	Opaque
MLT	transparent	Opaque
CIT	transparent	Opaque
PAC	transparent	Opaque
OX	see oxidase package insert	

A. Negative tests

B. Positive tests

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 408, Oxidase Test
4. SOP# 404, Gram Staining Procedure
5. SOP# 407, Bacterial Identification Using API 20 N
6. SOP 410, Presumptive Bacterial Identification Procedures
7. SOP# 411, Flowchart for the Presumptive Identification of Bacterial Fish Pathogens
8. Package insert for API 20 NE
9. Biomerieux website: https://apiweb.biomerieux.com/

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Oxidase Test**	
SOP #: 408	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods to conduct an oxidase test, which helps to aid in the identification of Gram negative, non-fermenting bacteria. The oxidase test helps to determine if an organism is aerobic based on the presence of the electron transport chain which is the final phase of aerobic respiration

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic[2].
- Use aseptic technique when working with live bacterial cultures.
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIALS REQUIRED

1. BD oxidase reagent stain droppers (0.5 mL)
2. Inoculating loop
3. Filter paper sheets or swabs
4. Bacterial sample to be tested

E. PROCEDURES

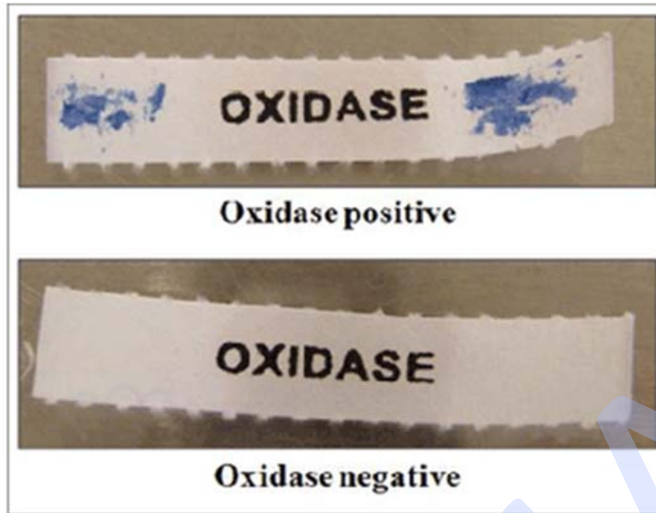
1. Take one ampule of oxidase reagent and holding the dropper upright, squeeze outer plastic tube firmly until the glass ampule inside breaks.
2. Turn the dropper upside down and add 5 drops of the reagent directly to the filter paper or swab.
3. Using a sterile loop, collect an isolated bacterial colony.
4. Smear the isolated bacterial colony onto the filter paper or swab where the oxidase reagent was placed.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

A positive reaction will turn violet/purple within 30 seconds. See photographs below.



Flow chart of Procedures of Oxidase Test

Isolate from pure culture

- Filter paper method → Soak filter paper in reagent solution → Scrape fresh growth using loop / stick and smear on filter paper or Touch a colony with edge of paper → Observe colour changes within 10s
- Direct plate method → Add 2-3 drops of reagent to colony on plate → Observe colour changes within 10s
- Swab method → Dip swab in the reagent and then touch colony → Observe colour changes within 10s
- Impregnated oxidase strip method → Scrape fresh growth using loop / stick and smear on filter paper → Observe colour changes within 10s
- Test tube method → Add 0.2mL of 1% naphthol and 0.3mL of 1% p-aminodimethylaniline oxalate into overnight broth culture → Observe colour changes within 10-30s

Appearance of deep purple-blue colour → Positive

No colour change → Negative

www.microbiologyinfo.com

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. American Fisheries Society-Fish Health Section (AFS-FHS) Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens (most recent edition). Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php .

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Motility Test**	
SOP #: 409	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes multiple methods used to determine if a bacterial isolate is motile by means of flagella.

B. DEFINITIONS

1. Brownian motion - the erratic random movement of microscopic particles in a fluid, as a result of continuous bombardment from molecules of the surrounding medium.

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic[2].
- Use aseptic technique when working with live bacterial cultures.
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Phosphate buffered saline (PBS)[3]
2. Hanging drop slide
3. Cover slips
4. Microscope
5. Inoculating loops or sterile swabs
6. Immersion oil
7. 5 mm^2 block of agar
8. Bacterial isolate(s) in question (grown on appropriate media)

E. PROCEDURES

1. Hanging drop method:
   - Inoculate a tryptic soy agar (TSA[4]) plate or tryptic soy broth (TSB[5]) with the bacterial isolate. Note: Use suitable medium for those organisms that do not grow on TSA or in TSB (e.g., yellow pigmented organisms can be grown on TYES[6]).
   - Incubate at room temperature (or other appropriate temperature) until growth is obtained, usually 24 hours.
   - For isolates grown on agar, place a drop of PBS onto the center of a clean coverslip. Inoculate the center drop with pure bacterial strain culture using a sterile loop. For isolates grown in broth, use a sterile loop or sterile dropper and place a drop in the center of a clean coverslip.
   - Carefully invert the coverslip and place over the concave portion of a hanging drop slide.
   - Observe for motility at 400x (40x objective and 10x ocular lens) magnification on a compound microscope. Care should be taken to not interpret “drift” or “Brownian motion” as motility.

  - Record results as motile or non-motile. Note: If the hanging drop slide was prepared from an isolate grown on agar and the bacterium appears to be non-motile, an additional hanging drop test using TSB (or other suitable broth medium) or semi-solid medium method must be done to confirm true non-motility.
2. Agar block method for Flavobacteria:
   - Excise a 5-mm square block of agar supporting a suspected flavobacteria colony.
   - Place the block (colony side up) on a glass slide, and cover the block with a cover glass.
   - Examine the colony edge with a microscope at about 400x (40x objective and 10x ocular lens) magnification for evidence of gliding or creeping motility.

F. QUALITY CONTROL

- Take care to ensure no cross contamination between bacterial isolates to be tested.
- Use *Escherichia coli* (ATCC 25922) for a motile positive control, and *Aeromonas salmonicida* for a negative control.

G. INTERPRETATION

1. Hanging drop method: Observe for motility at 400x (40x objective and 10x ocular lens) magnification on a compound microscope. Care should be taken to not interpret "drift" or "Brownian motion" as motility.
2. Agar block method for Flavobacteria: Examine the colony edge with a microscope at about 400x (40x objective and 10x ocular lens) magnification for evidence of gliding or creeping motility.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 821, Phosphate Buffered Saline (PBS)
4. SOP# 837, Tryptic Soy Agar (TSA)
5. SOP# 836, Tryptic Soy Broth (TSB)
6. SOP# 838, Tryptone Yeast Extract Salts Agar (TYES)
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Presumptive Bacterial Identification Procedures**	
SOP #: 410	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes the methods for presumptive identification of bacterial species recovered from fish.

B. DEFINITIONS

N/A

C. SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment when handling reagents and live bacterial cultures[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. All materials needed in Gram Staining Procedure[2]
2. All materials needed in Potassium Hydroxide Gram Reaction[3]
3. All materials needed in Oxidase Test[4]
4. All materials needed in Bacterial Identification Using API 20E[5]
5. All materials needed in Bacterial Identification Using API 20NE[6]
6. All materials needed in *Flavobacterium psychrophilum* Direct Fluorescent Antibody Test[7]
7. All materials needed in *Renibacterium salmoninarum* Direct Fluorescent Antibody Test[8]
8. Flowchart for bacterial identification[9]

E. PROCEDURES

1. After culturing from appropriate tissues[10,11] examine cultures daily and record growth on worksheet.
   - Colonies for identification should exhibit morphology of known pathogens, be in large numbers, or be of pure culture.
   - Sometimes the initial isolate is a rapid growing opportunist that masks the pathogen.
2. If pure colonies are not distinguishable, select a single mixed colony and streak to a separate plate for isolation.
   - Mixed contamination should not be isolated and identified unless requested by the pathologist.
3. From the same or an identical colony, make a Gram stain.
   - If deemed necessary by Fish Health Supervisor, perform further tests.
4. Using results from aforementioned biochemical tests, follow through the Flowchart for Bacterial Identification.
5. Report all presumptive results to the Fish Health Supervisor.
6. Continue to confirmatory tests if needed.

F. QUALITY CONTROL

- Use aseptic technique when handling and processing all samples, bacterial cultures, and reagents.

G. INTERPRETATION

Report all results to Fish Health Supervisor.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 404, Gram Staining Procedure
3. SOP# 405, Potassium Hydroxide Test for Gram Reaction
4. SOP# 408, Oxidase Test
5. SOP# 406, Bacterial Identification Using API20 N
6. SOP# 407, Bacterial Identification Using API20 NE
7. SOP# 415, *Flavobacterium psychrophilum* Direct Fluorescent Antibody Test
8. SOP# 414, *Renibacterium salmoninarum* Direct Fluorescent Antibody Test
9. SOP# 411, Flowchart for the Presumptive Identification of Bacterial Fish Pathogens
10. SOP# 302, General Necropsy and Sample Collection for Inspections
11. SOP# 303, Field Collection Of Bacteriology Samples
12. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached data log.

ORIGINAL

Diagnostics: Bacterial Growth Tracking Sheet

Sample Site:	
Date Collected:	
Received From	
Fish Examined	

Case Number:	
Date Received Lab:	
Received By	
Sample Condition	

Sample Processing

Date Cultured	Tissue Cultured	Media

Date	Growth	Comments/Tech

Diagnostic Procedures/Tests

Date	Test/Stain	Comments/Tech

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Flowchart for the Presumptive Identification of Bacterial Fish Pathogens**	
SOP #: 411	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) presents a dichotomous key that may be used for the presumptive identification of bacterial fish pathogens using biochemical tests. Portions of this SOP were derived directly from listed associated documents.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. N/A

E. PROCEDURES

1. See flowcharts below.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

Follow flowcharts below and report all results to Fish Health Director.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. American Fisheries Society-Fish Health Section (AFS-FHS) Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens (most recent edition). Accessible at: http://afs-fhs.org/bluebook/bluebook-index.php .

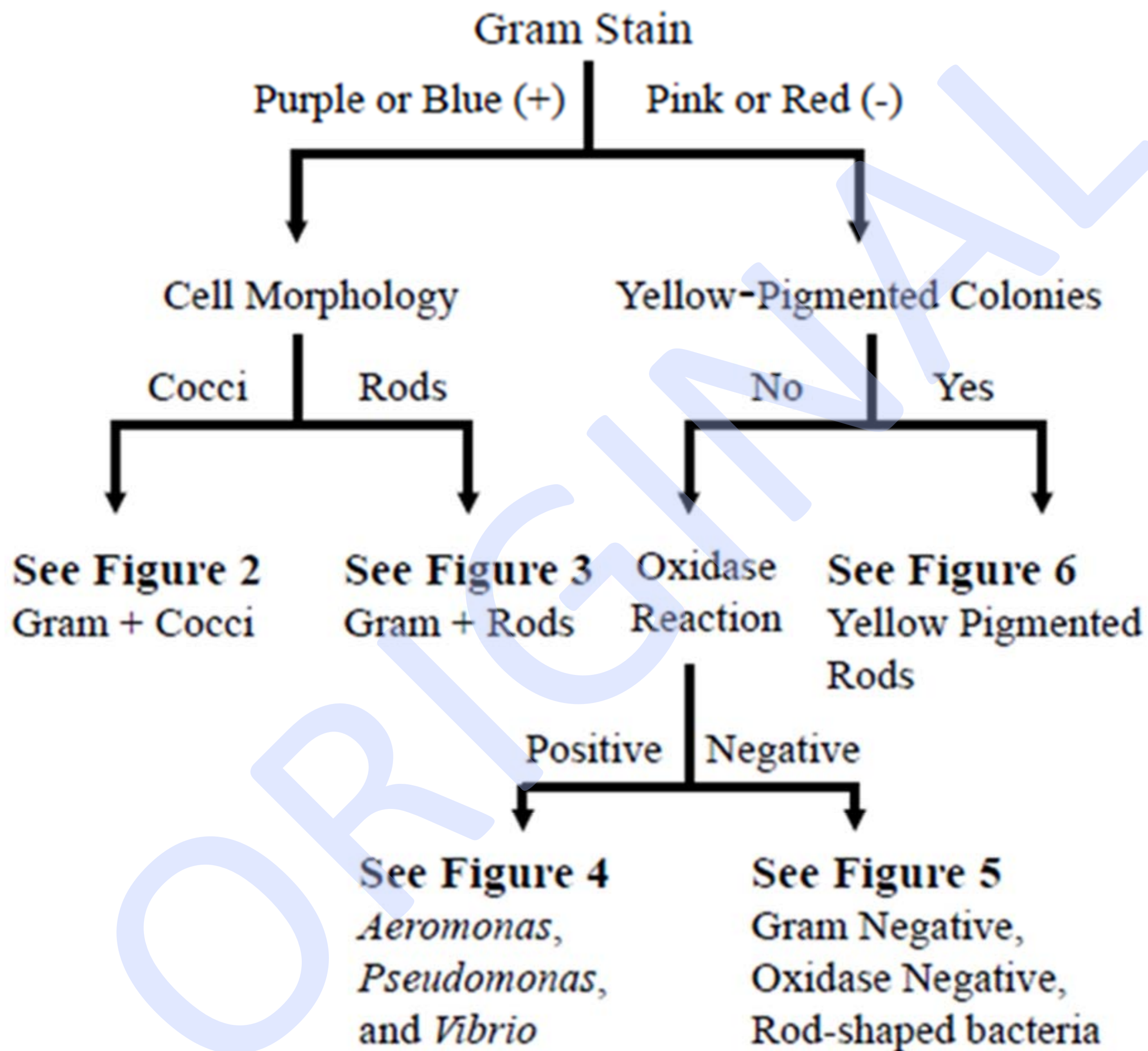
I. REVISION HISTORY

1. Original

ORIGINAL

J. APPENDICES

- Flowcharts for the presumptive identification of bacterial fish pathogens.



ORIGINAL

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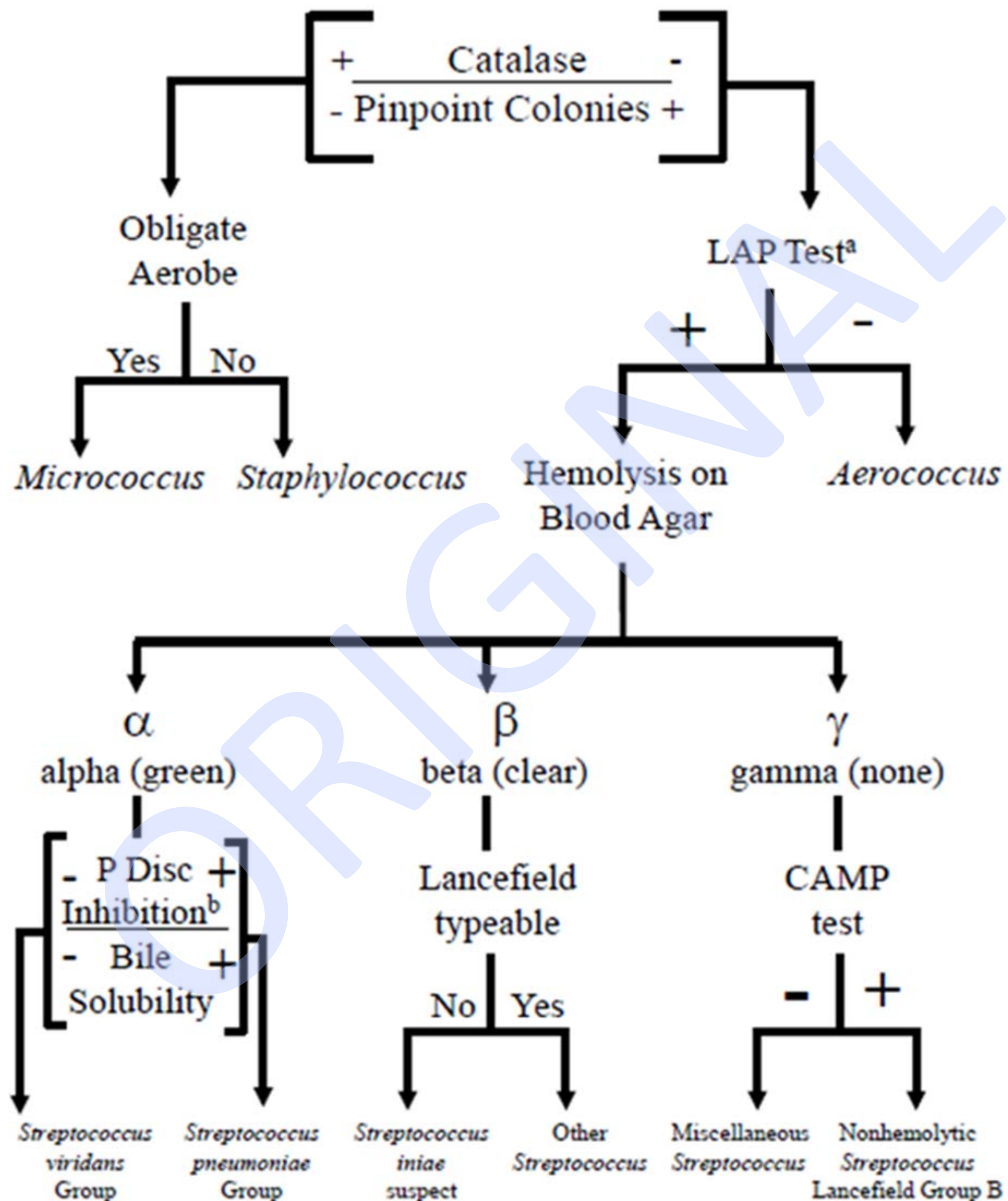
Figure 2: Gram Positive Cocci



a = leucine aminopeptidase test
b = ethyl hydrocuprein hydrochloride (5 µg) disc

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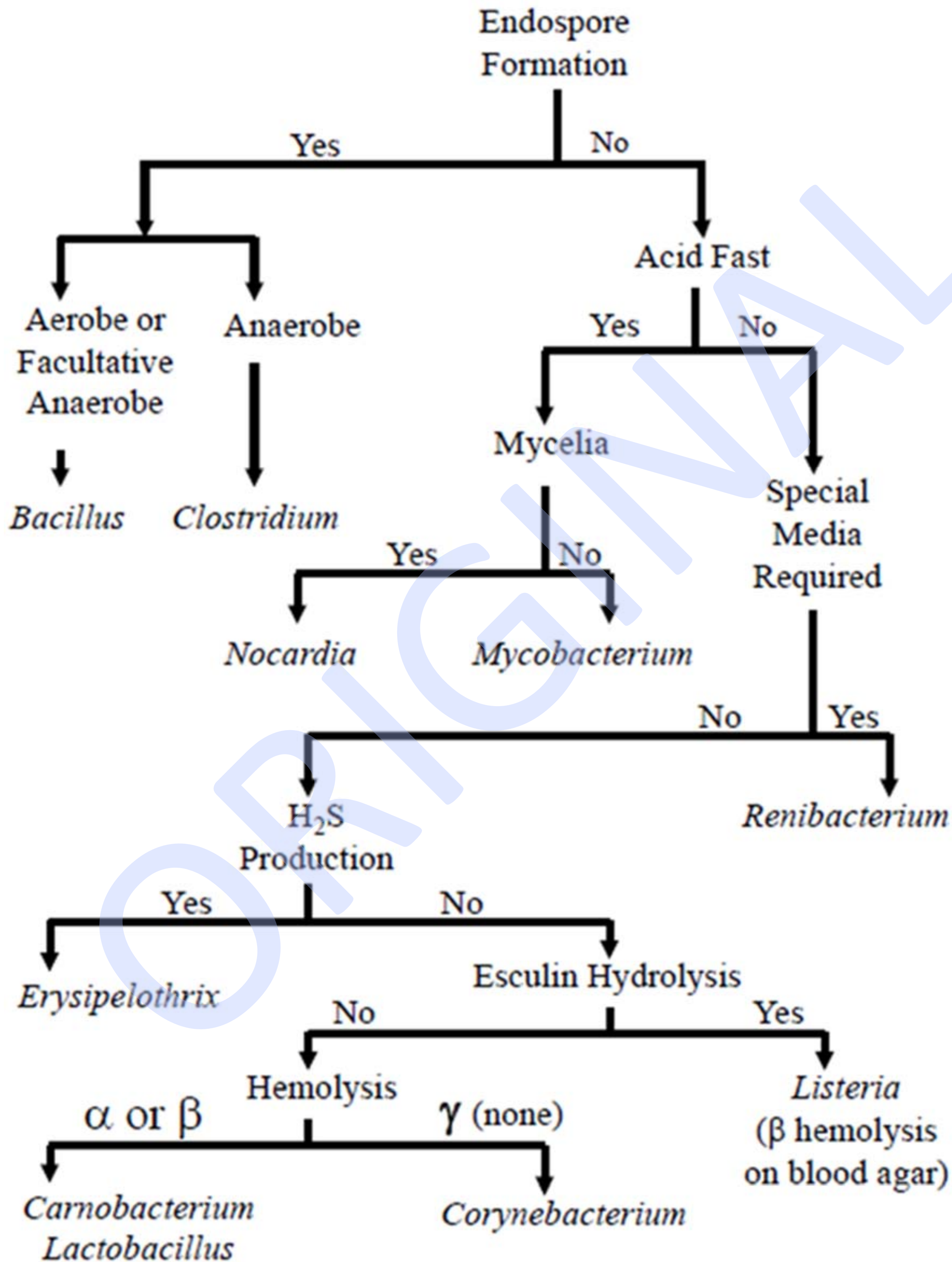
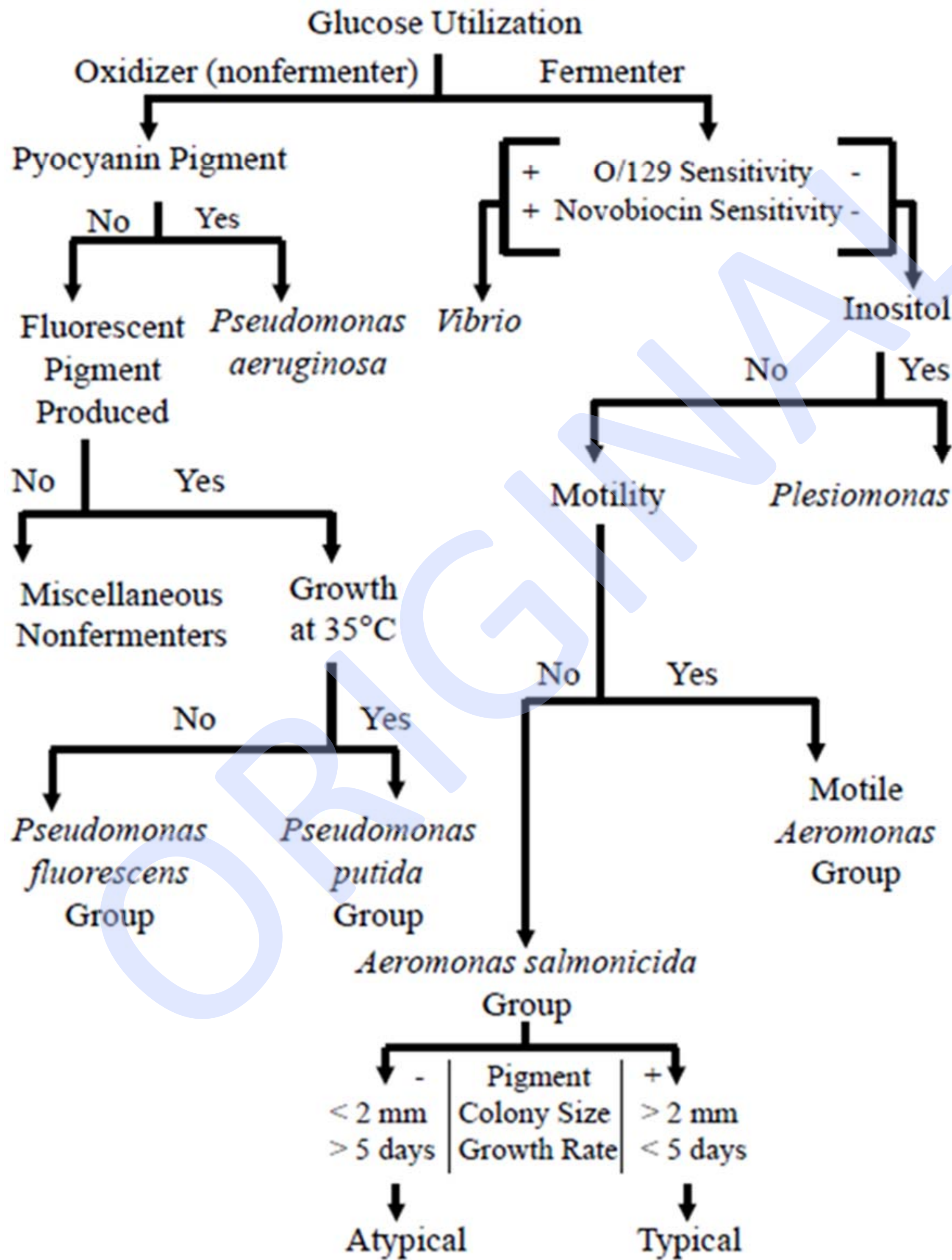
ORIGINAL

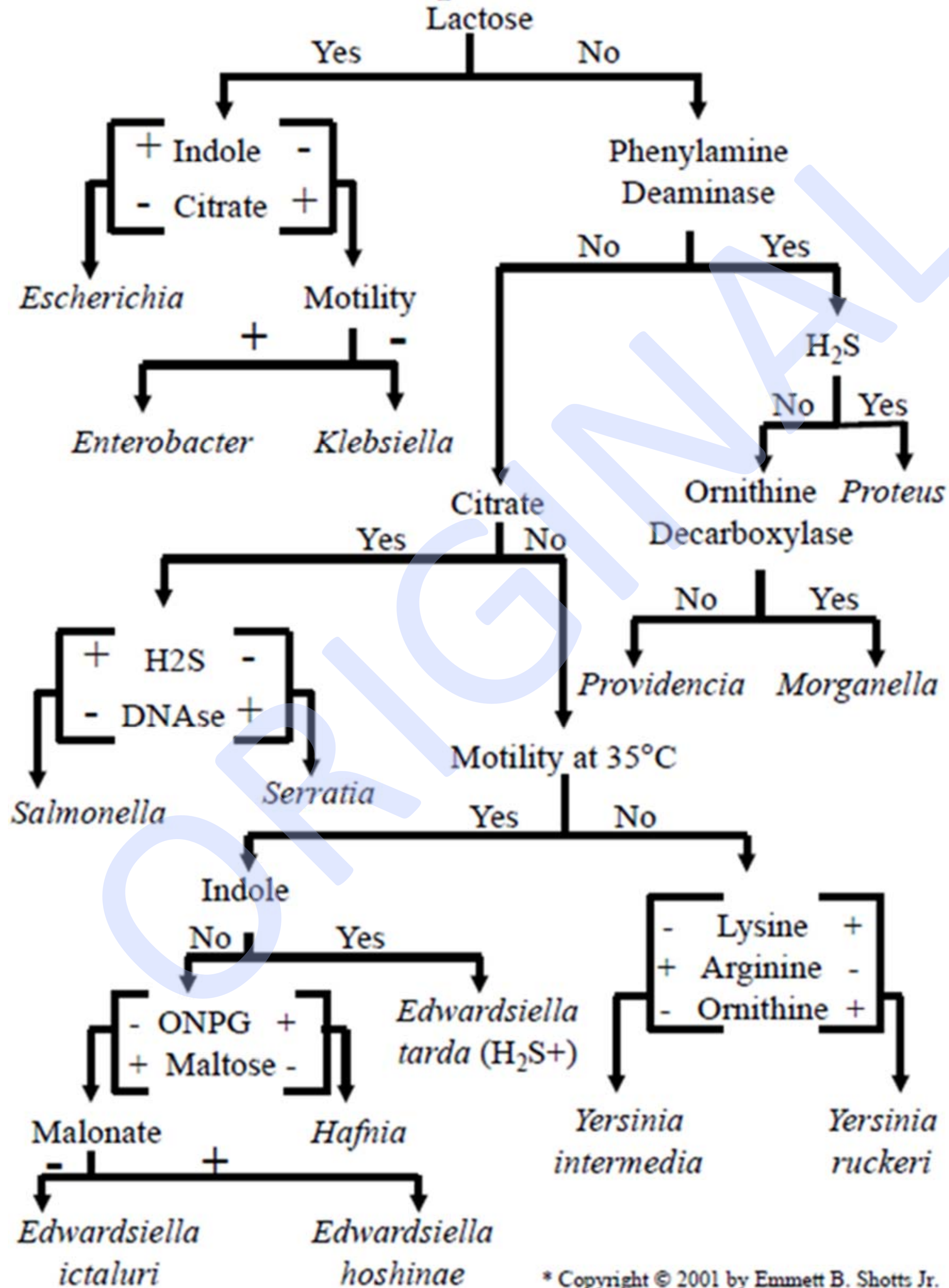


ORIGINAL



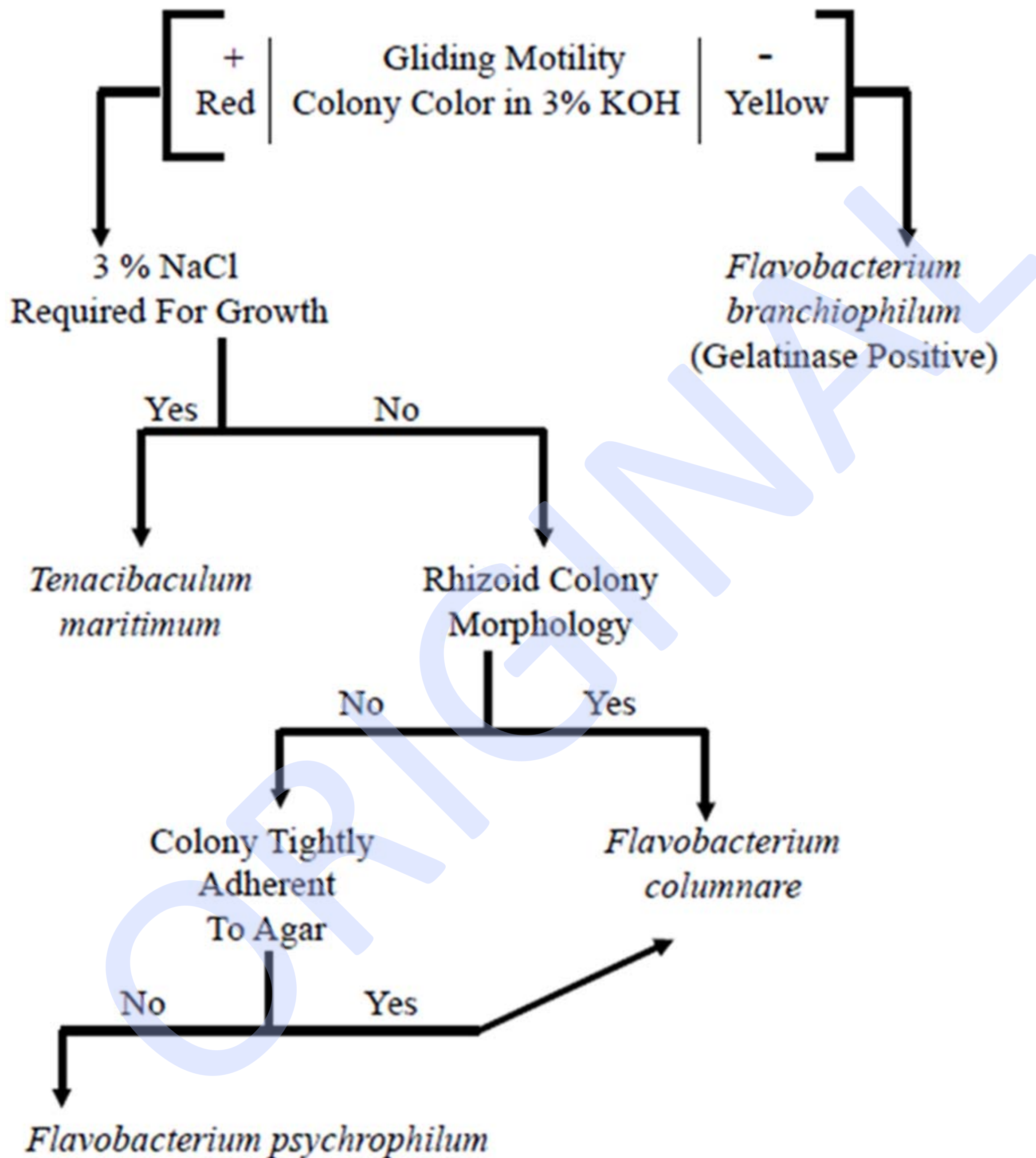
*Copyright © 2001 by Emmett B. Shotts Jr.

ORIGINAL



ORIGINAL

Figure 6: Yellow-Pigmented Rods[1]



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[1] New *Flavobacterium* spp. and related yellow-pigmented bacteria have been described complicating this flow chart. Definitive identification using molecular techniques may be necessary.

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§Updated 2014 by Ben LaFrentz

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Antibiotic Disc Sensitivity Testing**	
SOP #: 412	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes a standardized method for determining the antimicrobial susceptibility of *Aeromonas salmonicida* on Mueller-Hinton, tryptic soy agar (TSA) or tryptone yeast extract salts agar (TYES), *Flavobacterium psychrophilum* on TYES agar and for other common bacteria on TSA, TYES, or Mueller-Hinton agar using the disc diffusion method. Disc diffusion testing indicates susceptibility of a bacterium to an antibiotic by the presence of a clear zone of inhibited growth around an antibiotic impregnated paper disc placed directly on an agar plate that has been coated with a known concentration of a bacterial culture. The diameter of the inhibited growth zone allows for the bacteria to be categorized as susceptible, intermediate or resistant to a specific antibiotic based on pretested, known guidelines.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures[2].
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIAL REQUIRED

1. TYES agar plates[3]
2. TSA agar plates[4]
3. Mueller-Hinton agar plates[5]
4. 12 x 75mm disposable culture tubes
5. Parafilm
6. Sterile phosphate buffered saline (PBS)[6]
7. Cotton tipped applicators
8. Disc dispenser
9. Inoculation loops
10. Toothpicks
11. Log phase cultures on agar plates
12. McFarland standards
13. Sensitivity discs
    - Penicillin 10
    - Florfenicol 30 µg
    - Oxytetracycline 30 µg
    - Erythromycin E15

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E. PROCEDURES

1. Preparation of reagents and media:
   - Prepare PBS according to manufacturer's instructions using deionized water. Sterilize as directed.
   - Prepare agar according to manufacturer's instructions or lab protocols using deionized water. Sterilize as directed.
   - Dispense agar into plates to a thickness of about 4mm. Cover plates and let agar set until hard. Invert plates, place in sleeves and refrigerate.
2. Bacterial culture:
   - Incubate bacteria to be tested at an appropriate temperature to produce fresh log phase growth cultures (generally 15ºC or room temperature for most bacteria in this lab).
   - Streak plates so as to obtain distinct separate colonies for isolation and testing.
3. Sample collection:
   - Harvest isolated bacterial colony(ies) from log phase growth on plate culture.
   - Suspend colony(ies) in sterile PBS to 0.5 - 1.0 McFarland standard depending on bacteria to be tested (0.5 for most bacteria but *F. psychrophilum* can be suspended to a 1.0 standard).
4. Innoculation of sensitivity plates:
   - Take a sterile cotton tipped applicator and immerse it in the pre-suspended PBS/bacterial solution until completely saturated.
   - Remove cover from agar plate and apply the applicator to the plate smearing the PBS/bacteria mixture across the entire plate surface. Turn the plate 45 degrees and repeat the process in this direction to ensure even coverage of the mixture over the entire plate, rotate and repeat one more time. Cover plate.
5. Application of sensitivity discs:
   - Remove cover from agar plate and place plate on a flat stable surface. Place disc dispenser on top of plate and pull lever to release sensitivity discs (dispenser is stored under refrigeration).
   - Using a new toothpick for each disc, press down on each disc to make sure they adhere to the surface of the agar.
   - Cover plate, label, seal with parafilm, invert and store at the appropriate temperature.
   - Check plates daily for growth (may be up to 3 days before growth is seen on *F. psychrophilum* plates.

F. QUALITY CONTROL

- Consider all bacteria to be pathogenic.
- Sterilize your equipment and materials.
- Use aseptic technique.
- Label everything clearly.
- Disinfect all work areas before and after working with bacteria.
- Wash your hands frequently.
- Autoclave wastes as soon as possible.
- Clean up any spills immediately and carefully.
- Do not EVER pipette anything by mouth.
- Do not bring food or drink into the lab.

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G. INTERPRETATION

See tables below.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 838, Tryptone Yeast Extract Salts Agar (TYES)
4. SOP# 837, Tryptic Soy Agar (TSA)
5. SOP# 818, Mueller Hinton Agar (MH)
6. SOP# 821, Phosphate Buffered Saline (PBS)
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
8. Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Proposed Guideline, Document M42-P developed by the Clinical and Laboratory Standards Institute

I. REVISION HISTORY

1. Original

J. APPENDICES

- **Table 1: Interpretation of zone diameter for *Aeromonas salmonicida* on Mueller-Hinton, TSA or TYES Agar incubated at room temperature for 24-48 hours.**

Antibiotic Disk concentration	Florfenicol 30 µg	Oxytetracycline 30 µg	Ormetoprim/sulfa 1.2 / 23.8 µg	Ampicillin 10 µg
Resistant	16 mm	14 mm	11 mm	13 mm
Intermediate	17-20 mm	15-18 mm	12-16 mm	14-16 mm
Sensitive	21 mm	19 mm	17 mm	17 mm

*Zone diameter categories are taken from package inserts provided with the discs

- **Table 2: Interpretation of zone diameter for *Flavobacterium psychrophilum* on TYES agar incubated at 15°C for 48-72 hours.**

Antibiotic Disk concentration	Florfenicol 30 µg	Oxytetracycline 30 µg	Ormetoprim/sulfa 1.2 / 23.8 µg	Ampicillin 10 µg
Resistant	16 mm	14 mm	Used on TYES	13 mm
Intermediate	17-20 mm	15-18 mm		14-16 mm
Sensitive	21 mm	19 mm		17 mm

*Zone diameter categories are taken from package inserts provided with the discs

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- **Table 3: Interpretation of zone diameter for common bacteria on Mueller-Hinton or TYES Agar. This table is based on NWFHS Laboratory Procedures Manual – Second Edition, June 2004 Chapter 5 – Page 24.**

Antimicrobial	Disc Content	Resistant	Intermediate	Sensitive
Erythromycin	15ug	No zone	<15mm	≥15mm
Novobiocin	30ug	No zone	<10mm	≥10mm
Oxolinic Acid	2ug	No zone	<15mm	≥15mm
Oxytetracycline	30ug	No zone	<15mm	≥15mm
Penicillin G	10U	≤11mm	12-21mm	≥22mm
Romet 30	25ug	No zone	<15mm	≥15mm
O/129	0.1% (W/V)	No zone	<7mm	≥7mm

Comparision of MacFarland Turbidity Standards to optical density (OD) measured at 525nm (these figures will differ with species). Experimentally derived using *Flavobacterium psychrophilum* bacteria mixed with PBS.

0.5 McFarland Standard ~0.020OD525
1.0 McFarland Standard ~0.056OD525
3.0 McFarland Standard ~0.246OD525

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Freezing of Bacterial Isolates**	
SOP #: 413	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods for preparing and freezing of bacterial isolates.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures[2].
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sterile 50:50 glycerol/PBS
2. Tryptone yeast extract salts (TYES[3]) broth or tryptic soy broth (TSB[4])
3. Pure bacterial isolates
4. 2.0 mL cryotubes
5. 50 mL centrifuge tubes

E. PROCEDURES

1. Isolate and confirm the bacteria to be saved using presumptive identification procedures.
2. Inoculate 20 mL of TYES (*Flavobacterium* spp.) or TSB (all other bacteria) in a sterile 50 mL centrifuge tube with a single well isolated colony.
3. Allow culture to grow to log phase (3-5 days for *Flavobacterium* spp. and 1-2 days for all other isolates).
4. Spin down at 3000 rpm for 5 minutes. Pour off all but approximately 5 mL media. Vortex to resuspend bacteria.
5. Mix 600 µL log phase bacterial culture with 400 µL glycerol:PBS (to obtain 20% glycerol) in a sterile 2.0 mL cryotube.
6. Mix well by vortexing and store at -80˚C.
7. Record the addition in the database.

F. QUALITY CONTROL

- Restreak bacterial culture and confirm using diagnostic methods.

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G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 838, Tryptone Yeast Extract Salts
4. SOP# 836, Tryptic Soy Broth

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Renibacterium salmoninarum* Direct Fluorescent Antibody Test**	
SOP #: 414	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods to detect the presence of *Renibacterium salmoninarum* in spleen and kidney samples by Direct Fluorescent Antibody Test (DFAT).

B. DEFINITIONS

- N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures[2].
- Disinfect all surfaces before and after working with any bacterial cultures.
- **Methanol-potential health hazards:**
  - **Skin:** Toxic in contact with skin. Skin absorption may cause toxic effects similar to those described for inhalation. Repeated or extended contact may cause erythema (reddening of the skin) or dermatitis resulting from a defatting action on tissue.
  - **Eyes:** Irritating to eyes. Symptoms include itching, burning, redness and tearing. Prolonged or acute contact may cause eye damage. This product may cause blindness if it is swallowed.
  - **Inhalation:** Toxic by inhalation. May cause blindness if inhaled. Vapors may cause drowsiness and dizziness. Inhalation of high vapor concentrations can cause CNS-depression and narcosis. Severe overexposure may produce more serious symptoms including coma and risk of liver damage.
  - **Ingestion:** Toxic if swallowed. May be fatal or cause blindness if swallowed. Ingestion of this product may result in central nervous system effects including headache, sleepiness, dizziness, slurred speech and blurred vision.
  - **Delayed effects:** Repeated or prolonged exposure may cause damage to the liver and central nervous system. This product may cause adverse reproductive effects. Methyl alcohol can produce damage to the optic nerve and central and motor nerves.
  - **Medical conditions aggravated by exposure:** Pre-existing liver dysfunctions or eye, skin and/or central nervous system disorders may be aggravated by exposure.
- **Rhodamine-potential health hazards:**
  - **Skin:** Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion and of inhalation.
  - **Potential Chronic Health Effects:** Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion and of inhalation.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 12x75mm culture tubes

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2. Sterilized wooden applicator sticks
3. Autoclave bags
4. *Renibacterium salmoninarum* positive samples stored in -80°C freezer (or premade positive control slides)
5. Tissues to be tested (usually stored in ethanol)
6. Multi-Spot Slides
7. Methanol
8. Cardboard popup slide holder that holds 20 slides
9. Rhodamine B[3]
10. Antibody conjugate for *Renibacterium salmoninarum*[4]
11. 1.5mL centrifuge tubes
12. 10mL syringe
13. 18 gauge needle
14. Phosphate buffered saline (PBS)[5]
15. Syringe 0.45µm Filter
16. Tips
17. Toothpicks
18. PBS squirt bottle
19. Aluminum foil
20. FA Mounting Fluid
21. Microscope cover glass
22. Immersion oil

E. PROCEDURES

1. Preparing slides:
   - Collect samples as directed in General Necropsy[6] and Field Collection of *Renibacterium salmoninarum* DFAT Samples[7].
   - Prepare all reagents needed in this SOP, including antibody and rhodamine. *Note, work in a dark room when handling these reagents as light will quench the fluorescence. Keep reagents in a dark box at 4°C until ready to use.
   - Prepare lab bench with butcher paper and appropriate biohazard waste containers.
   - Label with pencil 12-well multi-spot slides for the appropriate number of samples (a case of 60 fish will require 5 full slides).
   - Using a new sterile wooden stick for each sample, carefully remove the kidney sample from the tube/ethanol it was stored in. Lightly touch the tissue sample to the appropriate well.
   - Replace the sample back in the tube and break off the wooden stick in the tube, leaving a portion of the stick in the tube.
   - With the remainder of the wooden stick, smear the kidney sample around the well until it is evenly coated. Use care not to apply too much sample to the well as this makes viewing the samples difficult. Discard wooden stick.
   - Repeat until all samples have been smeared onto a slide.
   - Air dry and heat fix the slides.
   - Soak the slides in methanol for 10 minutes. Soaking in methanol removes lipids from the smears and increases fluorescence quality and intensity.
   - Remove slides from methanol and air dry.

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- Both positive and working samples should be prepared in this manner but at different times. You will need one positive slide for each case or for the numerous cases that are stained at the same time.

2. Staining slides with antibody reagents:
    - Turn off lights in the lab so you have a dark working area. Light will quench fluorescence on the slides.
    - On each well of the multi-spot slides, add 20 µL of the prepared fluorescein labeled affinity purified antibody to *Renibacterium salmoninarum*/rhodamine mixture.
    - Smear the mixture evenly over the well with a toothpick. Repeat with all 12-wells (using a new toothpick for each well).
    - Place the slide in a dark area.
    - Repeat with the remaining samples until all slides are stained.
    - Remove a positive control slide from the refrigerator and stain as above. Use a new tip for each sample so as not to contaminate the fluorescein/rhodamine stain.
    - Place the positive slide in a dark area.
    - Incubate for 1 hour at room temperature.
    - Rinse the working samples one at a time with a squirt bottle of PBS.
    - Place slides in a Coplin jar and soak in PBS 10 minutes.
    - Repeat with the positive control using a different Coplin jar.
    - Remove from PBS and allow to air dry in a cardboard slide holder until completely dry.
    - Place the positive control slide in the bottom right slot of the slide holder.
3. Reading slides:
    - Add a drop of FA mounting fluid to each well on the bottom row of the slide. Do not touch the dropper to the slide to prevent the contamination of the mounting fluid bottle.
    - Place a cover slip on the slide by putting the bottom edge down first and laying the cover slip down, forcing the mounting fluid to flow upwards towards the upper wells.
    - Tap with your finger to ensure all wells have mounting fluid.
    - To remove excess mounting fluid, place the slide on its side on a paper towel. This will wick out the excess fluid.
    - Add a drop of oil to each well to be examined.
    - View at 1000x using the FITC epifluorescent filter.
    - Read the positive control slides first. This ensures that the slides are stained correctly and that the positive bacteria are fluorescing as they should. This slide can be used to help the examiner identify bacteria size, shape and manner of fluorescence.
    - Examine at least 50 fields in each well at 1000x.

F. QUALITY CONTROL

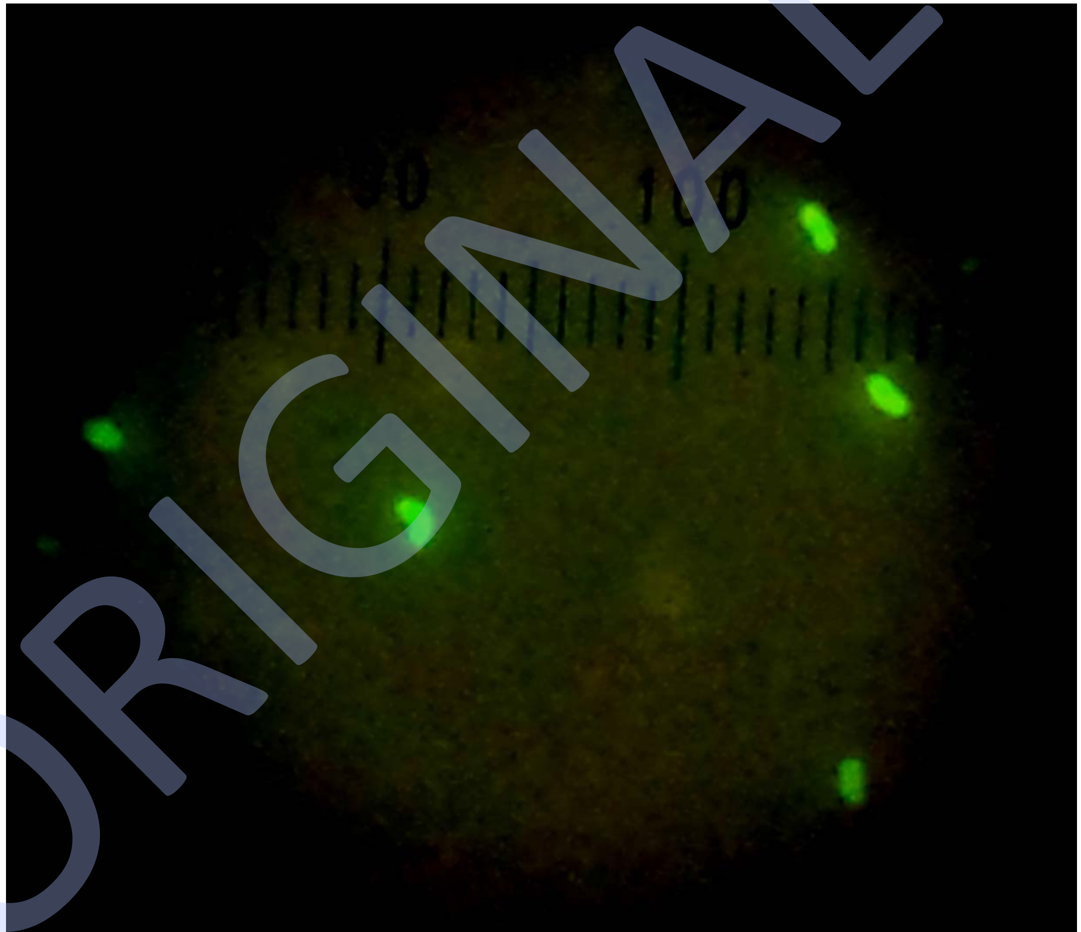
- Caution must be used when preparing positive control slides because it is extremely easy to contaminate surfaces and sample slides.
- The positive control slides should be prepared at some time other than when the working samples are being prepared.
- Numerous slides may be prepared at one time and stored, refrigerated, until such time as they will be stained.
- All bench top space, supplies and utensils should be thoroughly cleaned or disposed of after use.
- It is best to wear gloves when working with and viewing positive slides.
- Dispose of gloves prior to reading working samples.

ORIGINAL

- After viewing positive control slides on the microscope, the scope should be thoroughly cleaned with ethanol or isopropyl alcohol.

G. INTERPRETATION

Positive samples will fluoresce an apple green color and the positive bacterial cells will be about 1.0 x 0.5 µm. If there is no fluorescence then slides can be reported as negative. If fluorescence is noted on any slide with the typical 1.0 x 0.5 µm diplobacilli cells (see photograph below) being present then further testing (PCR) must be done to confirm *R. salmoninarum* is present.



ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 827, Rhodamine B
4. SOP# 808, Antibody Conjugate for *Renibacterium salmoninarum*
5. SOP# 821, Phosphate Buffered Saline (PBS)
6. SOP# 302, General Necropsy and Sample Collection for Inspections
7. SOP# 304. Field Collection of *Renibacterium salmoninarum* DFAT Samples

8. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Flavobacterium psychrophilum* Direct Fluorescent Antibody Test**	
SOP #: 415	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of *Flavobacterium psychrophilum* in spleen, kidney and skin blot samples by Fluorescent Antibody Test (FAT).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures and dispose of infectious materials[2] and hazardous wastes[3].
- Disinfect all surfaces before and after working with any bacterial cultures.

D. EQUIPMENT AND MATERIAL REQUIRED

1. Phosphate buffered saline (PBS)[4]
2. 0.5% (w/v) non-fat dry milk + PBS, pH 7.2 (NFDM-PBS)[5]
3. FITC conjugated MAb FL-43
4. *Flavobacterium psychrophilum* culture from agar plate
5. Samples collected from inspection or diagnostic case to be tested
6. Multi-spot slides
7. Bunsen burner
8. PBS squirt bottle
9. FA mounting fluid
10. Microscope cover glass
11. Immersion oil
12. Microscope with FITC filter

E. PROCEDURES

1. Sample collection:
   - Collect tissues for *F. psychrophilum* DFAT[5].
2. Preparation of reagents and antibody:
   - Prepare NFDM-PBS solution. Store in the refrigerator.
   - Dilute FITC conjugated MAb FL-43 1:100 in NFDM-PBS. Store in the refrigerator wrapped in tinfoil in a dark container.
3. Slide preparation and staining:
   - Prepare smears on 12 well 9mm multi-spot slides by dabbing select tissues directly on slide. Use care not to apply too much sample to the well as this makes viewing the samples difficult.

- Air dry and heat fix the slides.
- Add 50µL of the NFDM-PBS solution to each well.
- Incubate the slide in a humid chamber at room temperature for 15 minutes.
- Rinse slide with PBS using a squirt bottle.
- Apply 50µL FITC conjugated MAb FL-43-PBS mixture.
- Incubate the slide in a dark humid chamber for 30 minutes.
- Rinse slide with PBS using a squirt bottle.
- Dry slide in the dark.
- Prepare positive control slides as above. These can be prepared at the same time but use a separate slide for the positive controls.

4. Reading slides:
   - Add a drop of FA mounting fluid to each well on the bottom row of the slide. Do not touch the dropper to the slide to prevent the contamination of the mounting fluid bottle.
   - Place a cover slip on the slide by putting the bottom edge down first and laying the cover slip down, forcing the mounting fluid to flow upwards towards the upper wells.
   - Tap cover slip to ensure all wells are covered with mounting fluid.
   - To remove excess mounting fluid, place the slide on its side on a paper towel. This will wick out the excess fluid.
   - Add a drop of oil to the coverslip above each well to be examined.
   - View at 1000x (100x oil objective + 10x ocular) using the FITC epifluorescent filter.

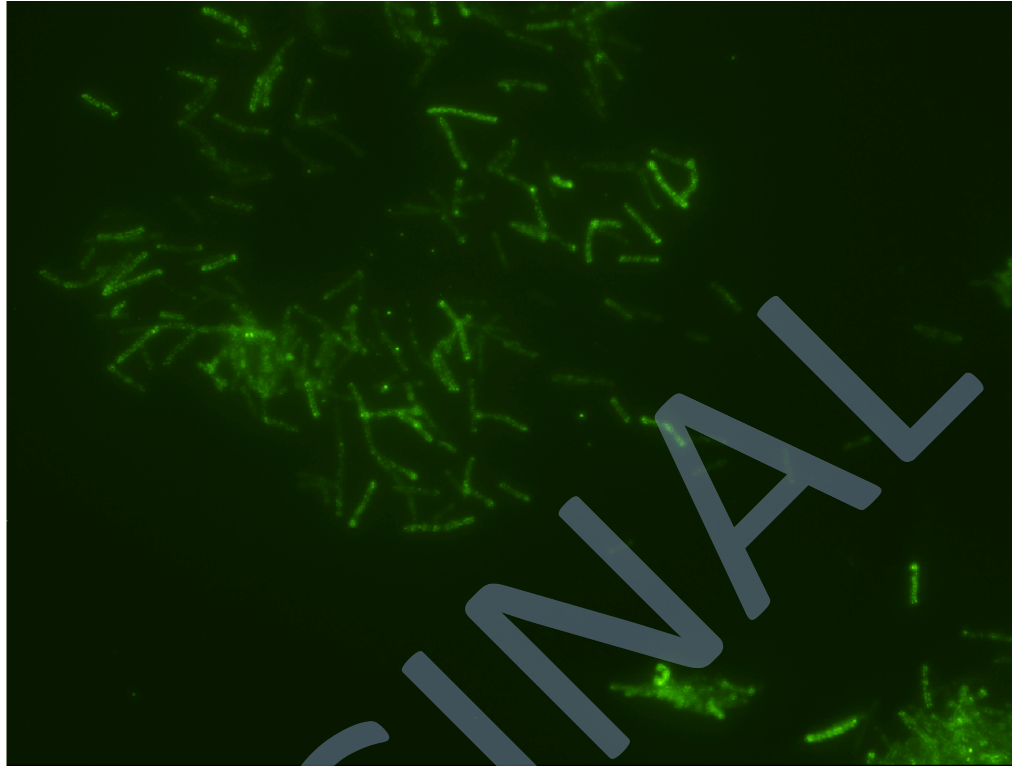
F. QUALITY CONTROL

- Positive control slides allow for identification based on morphology and verify that the bacteria are fluorescing correctly.
- All bench top space, supplies and utensils should be thoroughly cleaned with isopropyl alcohol or disposed of after use.
- It is best to wear gloves when working with and viewing positive slides.
- Dispose of gloves prior to reading working samples.
- After viewing positive control slides on the microscope the scope should be thoroughly cleaned with ethanol or isopropyl alcohol.

G. INTERPRETATION

Positive samples will fluoresce an apple green color and the positive bacterial cells will appear rather long and thin with a speckled or mottled appearance (see picture below). If fluorescence is noted on any slide with the typical long thin rods being present then *F. psychrophilum* is present. If there is no fluorescence then slides can be reported as negative.

ORIGINAL



***Flavobacterium psychrophilum* stained with FL-43 and viewed under epifluorescence.**

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal Of Infectious Agents
3. SOP# 209, Disposal Of Hazardous Wastes
4. SOP# 821, Phosphate Buffered Saline (PBS)
5. SOP# 823, Phosphate Buffered Saline With Tween 20 and Non-Fat Dry Milk (PBS-T20 + NFM)
6. SOP# 305, Field Collection of *Flavobacterium psychrophilum* Direct Fluorescent Antibody Test (FAT) Sample
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Flavobacterium psychrophilum* Bacterial Challenge**	
SOP #: 416	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes a standardized method for challenging fish with live *Flavobacterium psychrophilum*.

B. DEFINITIONS

1. MS-222: Tricaine methanesulfonate, used for fish anesthesia/euthanasia.
2. PBS: Phosphate buffered saline
3. TYES: Tryptone yeast extract salts media
4. MAT broth: Maltose and acetate supplemented tryptone yeast extract salts broth
5. FAT: Fluorescent antibody test
6. OD: Optical density

SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures.
- Disinfect all surfaces before and after working with any bacterial cultures and dispose of properly[2].
- Use caution when handling MS-222 and fish treated with MS-222 and dispose[3] of properly.

C. EQUIPMENT AND MATERIAL REQUIRED

1. Butcher paper
2. Bleach
3. Booties
4. Mop
5. Buckets
6. 70% Alcohol spray bottles
7. Paper towels
8. Trash bags
9. Tape
10. Nets
11. Scale
12. Measuring board
13. 1 mL syringes
14. Needles (25G)
15. Hematocrit tubes
16. Sharps container
17. Sterile phosphate buffered saline (PBS)
18. Inoculation loops
19. MS-222

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20. TYES agar plates
21. Parafilm
22. Frozen 259-93 stock
23. 50 mL MAT broth
24. 1 L MAT broth
25. Foam bottle closures
26. Aluminum foil
27. Gram slides
28. Stir bars
29. Stir plate
30. Incubator set to 15ºC
31. Centrifuge
32. Vortexer
33. Spectrophotometer
34. Pipettes to resuspend bacteria

D. PROCEDURES

1. Preparation of reagents and media:
   - Prepare PBS[4] and sterilize as directed.
   - Prepare TYES agar according to lab protocols[5] using deionized water and sterilize as directed.
   - Prepare MAT broth according to lab protocols[6]; you will need both a 50 mL flask and a 1.0 L flask of broth.
   - Place broth in incubator at 15ºC.
2. Bacterial culture:
   - Retrieve frozen stock of *F. psychrophilum* (strain 259-93) from -80°C. Allow to partially thaw at room temperature.
   - Streak TYES plates with loop of partially thawed *F. psychrophilum* culture so as to obtain distinct separate colonies for isolation and testing.
   - Incubate at 15ºC for approximately 72 hours.
   - Select a distinct colony from the plate, Gram stain[7] and run a FL-43 FAT to confirm *F. psychrophilum*[8].
     - Inoculate 50 mL of 15°C MAT broth with a loop of *F. psychrophilum* (strain 259-93) from a 72 hour culture on TYES plates. Place foam stopper inside flask opening and cover with aluminum foil.
     - Place on stir plate inside 15ºC incubator and stir very slowly for approximately 72 hours.
   - Aseptically remove 10 to 20 mL from the starter culture that has been growing for approximately 72 hours and dispense into 1.0 L flask of 15°C MAT broth.
     - Place foam stopped inside flask opening and cover with aluminum foil.
     - Place on stir plate inside 15ºC incubator and stir very slowly, for approximately 72 hours.
   - If bacterial culture appears turbid, then proceed to next step. If culture remains transparent after 72 hours, repeat previous steps in new media.
   - Aseptically transfer turbid bacterial culture to 2-4 50 mL conical tubes or larger sterile containers and centrifuge at 5000 rpm for 20 minutes.
     - Store remaining broth culture at 15°C until entire procedure is completed and it is not needed anymore.

ORIGINAL

- Resuspend the centrifuged bacterial culture in sterile PBS to an OD of 0.4 at 525 nm. Ensure preparation of a large enough volume at the appropriate OD for the number of fish to be inoculated.
- Fill 1 mL syringes fitted with appropriate sized needles with resuspended bacterial culture and keep refrigerated until ready to use. Bacterial will settle out so vigorously shake syringes to resuspend.
  - Ensure no air bubbles are in syringe.
- Prepare syringes with PBS for the negative control injections.

3. Preparation of challenge area:
   - Lay down butcher paper and/or large plastic garbage bags to cover table and tape down.
   - Clean and prepare buckets and nets that will be used. You will need both an MS-222 bucket and a recovery bucket.
   - Prepare mechanical air pump, hoses and air stones for both buckets.
   - Lay out length board, scale and sharps container.
   - Prepare a data form to note any information about individual fish or injections.
4. Blood and serum collection:
   - Follow protocol to collect blood and serum[9] from 20 fish at day zero.
5. Challenge:
   - Prepare MS-222 bucket for anesthetizing fish.
   - Remove a few fish from the target tank and place in MS-222 to assess dosage of MS-222.
   - Administer mock challenge to negative control fish using 50 µL of PBS. This is an intermuscular injection (IM) that should be administered to the side and slightly to the rear of the dorsal fin. Place fish in recovery bucket (fresh water) and return the entire group to the original labeled target tank following this procedure.
   - Maintain aeration in both MS-222 and recovery bucket during challenge period
   - Administer intermuscular injection (IM) to treatment groups using 50 µL of *F. psychrophilum* and follow procedure above.
   - Allow challenge to continue for at least 10 days, removing mortalities daily and record on data sheet.
   - Note water quality and water exchanges.
6. Blood and serum collection:
   - Follow protocol to collect blood and serum from remaining fish

ORIGINAL

E. QUALITY CONTROL

- Always include negative control fish/tanks in the study design. These fish will be inoculated with sterile PBS.
- Use aseptic technique during the procedure and every time when checking on fish.
- Always start with negative control fish first to reduce the risk of transferring pathogens from experimentally infected fish to negative control tanks.

F. INTERPRETATION

Assess days to death and relative percent survival (or mortality), as well as additional follow up regarding *F. psychrophilum* enzyme linked immunosorbent assays (ELISAs)[10].

G. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal Of Infectious Agents
3. SOP# 209, Disposal Of Hazardous Wastes

4. SOP# 821, Phosphate Buffered Saline (PBS)
5. SOP# 838, Tryptone Yeast Extract Salts (TYES) Media
6. SOP# 815, Maltose And Acetate Supplemented Tryptone Yeast Extract Salts (MAT) Broth
7. SOP# 404, Gram Staining
8. SOP# 415, Direct Fluorescent Antibody Test For *F. psychrophilum*
9. SOP# 417, Blood And Serum Collection
10. SOP# 418, *F. psychrophilum* Enzyme Linked Immunosorbent Assay (ELISA)
11. SOP# 817, Tricaine Methanesulfonate (MS-222) Preparation

H. REVISION HISTORY

1. Original

I. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Blood and Serum Collection from Fish**	
SOP #: 417	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods for collecting blood and serum from fish.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution when handling sharp needles[1].
- Do not use MS-222 on fish that may be stocked or consumed for food within 21 days.
- Wear protective clothing, gloves, and safety glasses when handling MS-222 powder[2].
- Limit the risk of inhalation[3].
- Wear gloves to handle water medicated with and animals exposed to MS-222[4].

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.0 mL syringes
2. 24 gauge needles (or appropriate sized needles for fish to be sampled)
3. Fish to be sampled
4. Tricaine methanesulfonate (MS-222)
5. New razor blades
6. Hematocrit tubes
7. 1.5 mL centrifuge tubes
8. 0.5 mL centrifuge tubes
9. Centrifuge
10. Pipettor
11. Pipette tips

E. PROCEDURES

1. Euthanize or anesthetize fish using MS-222.
   - See Tricaine Methanesulfonate (MS-222) for specifications.
2. When small fish are to be euthanized prior to blood collection:
   - Process fish immediately after euthanization to prevent blood clotting before collection.
   - Dry fish to minimize the possibility of water contacting the blood.
   - Use a new razor blade to remove tail at caudal peduncle.
   - Collect blood from the caudle vein and/or artery into a hematocrit tube.
   - Collect at least 200 µL of blood if possible.
   - Proceed to step 4.

ORIGINAL

3. When larger fish are to be anesthetized:
    - Insert a fresh needle attached to syringe into the caudle artery.
    - Pull back on syringe to create suction and collect blood.
    - Collect at least 200 µL of blood if possible.
    - Place fish into fresh water and monitor recovery.
    - Proceed to step 4.
4. Remove needle from syringe and express the blood into 1.5 mL centrifuge tubes.
5. Centrifuge blood at 4°C and 15,000 rpm for 5 minutes.
6. Collect serum using a pipettor.
7. Place serum into 0.5 mL centrifuge tubes and freeze at -80°C.

F. QUALITY CONTROL

- N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 205, Handling And Disposal Of Sharps
2. SOP# 201, Personal Protective Equipment
3. SOP# 206, Handling Of Hazardous Materials And Chemical Inventory
4. SOP# 817, MS-222, Tricaine Methanesulfonate

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Flavobacterium psychrophilum* Enzyme Linked Immunosorbent Assay**	
SOP #: 418	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods used to perform an enzyme-linked immunosorbent assay (ELISA) to detect rainbow trout antibody specific to *Flavobacterium psychrophilum* in fish serum or mucus.

B. DEFINITIONS

1. ELISA: Enzyme-linked immunosorbent assay
2. PBS: Phosphate buffered saline
3. MAb: Monoclonal antibody

C. SAFETY PRECAUTIONS

- Wear gloves when working with PBS-Azide.
- When mixing PBS-Azide work under the fume hood with appropriate safety clothing[1] and gear.
- Sodium Azide is very hazardous in case of skin contact or eye contact (irritant). It is hazardous in case of ingestion or inhalation. It is slightly hazardous in case of skin contact (permeator). Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering and itching. Skin inflammation is characterized by itching, scaling, reddening or occasionally, blistering.
- Sodium Azide is extremely reactive or incompatible with metals.

D. EQUIPMENT AND MATERIAL REQUIRED

1. ELISA plates: Immulon2 HB flat bottom microtiter plates
2. Coating antigen[2]
3. Primary antibody: Samples of fish serum or mucus to be analyzed
4. Positive control serum and/or mucus
5. Negative control serum and/or mucus
6. Secondary antibody: Mouse monoclonal antibody to trout immunoglobulin from hybridoma clone (WARR 1.14 MAb).
7. Tertiary antibody: Horseradish peroxidase-conjugated goat antibody to mouse immunoglobulin (HRP-conjugate, Calbiochem #401253)
8. Substrate: ABTS-peroxidase substrate (Kirkegaard and Perry Labs #50-64-02 and #50-65-02)
9. Stop solution 1.0% sodium dodecyl sulfate solution (SDS, BioRad #161-0310)
10. Wash solution: PBS-T (0.05% Tween 20)[3]
11. Carbonate coating buffer[4]
12. PBS – bulk recipe[5]
13. PBS Azide (0.02%)
14. PBS-T + non-fat dry milk[6]

ORIGINAL

15. Multichannel pipette(s)
16. 200 µL tips
17. 1-10 µL tips
18. Squirt bottles
19. Paper towels
20. Gloves
21. Timer
22. Plate reader
23. Templates for plate layout
24. Computer with GEN5 software
25. Epoch plate reader

E. PROCEDURES

1. Prepare reagents:
   - Prepare secondary antibody: mouse monoclonal antibody to trout immunoglobulin from hybridoma clone (WARR 1.14 MAb).
     - Dilute secondary antibody 1:400 in PBS-T (0.05% Tween 20) containing 0.1% non-fat dry milk.
   - Prepare tertiary antibody: Horseradish peroxidase-conjugated goat antibody to mouse immunoglobulin (HRP-conjugate).
     - Dilute tertiary antibody 1:5000 in PBS-T containing 0.1% non-fat dry milk.
   - Prepare carbonate coating buffer.
2. Lay out plates on copies of the provided templates (see attached plate layout).
   - Put layout information into the plate reader program GEN5[7] (EPOCH by BioTech).
3. Coating antigen:
   - Dilute antigen to10 µg/mL in carbonate coating buffer.
   - Add 100 µL per well.
   - Incubate overnight at 4ºC.
   - Wash 3 times.
4. Primary antibody:
   - Add serum samples 100 µL per well.
     - Unknown serum samples: Dilute in PBS-AZ (0.02%) over range desired.
     - Positive control serum: Dilute 1:200 in PBS-AZ (0.02%).
     - Negative control serum: Dilute 1:200 in PBS-AZ (0.02%).
     - Negative (-) WARR and negative (-) conjugate serum: Dilute 1:200 in PBS-AZ (0.02%).
   - Incubate overnight at 15ºC (you can incubate at 4ºC).
   - Wash 3 times.
5. Secondary antibody (WARR 1.14 MAb):
   - Add 100 µL per well.
   - Incubate 1 hour at room temperature.
   - Wash 3 times.
6. Tertiary antibody (HRP-conjugate):
   - Add 100 µL per well.
   - Incubate 1 hour at room temperature.
   - Wash 3 times.
7. Substrate reaction:

ORIGINAL

- Add 50 µL per well of ABTS peroxidase substrate + H_2O_2 (1:1).
- Incubate 15 minutes at room temperature.
- Add 50 µL of stop solution and read optical density at 405 nm.
- See attached example of plate readings.

F. QUALITY CONTROL

- See this ELISA guide for general quality control tips and for more background on ELISA techniques: https://www.idexx.com/pdf/en_us/livestock-poultry/elisa-technical-guide.pdf

G. INTERPRETATION

Determine positive-negative threshold. In many instances when doing an ELISA it is done in this fashion: calculate the average and standard deviation for the negative control wells. The threshold is the mean of the negative control + 2 standard deviations. In an effort to be very conservative in our determination of a positive "elevated" antibody titer we generally use a number that is 2 times the average of the two negative controls on each plate.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 810, *F. psychrophilum* ELISA Coating Antigen
3. SOP# 822, Phosphate Buffered Saline with Tween 20 (PBS-T20)
4. SOP# 804, Carbonate/Bicarbonate Coating Buffer
5. SOP# 821, Phosphate Buffered Saline (PBS)
6. SOP# 823, Phosphate Buffered Saline with Tween 20 and Non-Fat Dry Milk (PBS-T20 + NFM)
7. SOP# 419, ELISA Epoch Plate Reader And Gen5 Software Protocol
8. SOP# 417, Blood And Serum Collection
9. ELISA from BoLing Sun, University of Idaho, November 1998, revised April 1999 and revised by Ben LaFrentz April 2001
10. LaFrentz BR, LaPatra SE, Jones GR, Congleton JL, Sun B, Cain KD (2002) Characterization of serum and mucosal antibody responses and relative percent survival in rainbow trout (Oncorhynchus mykiss) following immunization and challenge with *Flavobacterium psychrophilum*. J Fish Dis 25:703–713
11. Swan CM, Lindstrom NM, Cain KD (2008) Identification of localized mucosal immune response in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following immunization with a protein-hapten antigen. J Fish Dis 31: 383-393

I. REVISION HISTORY

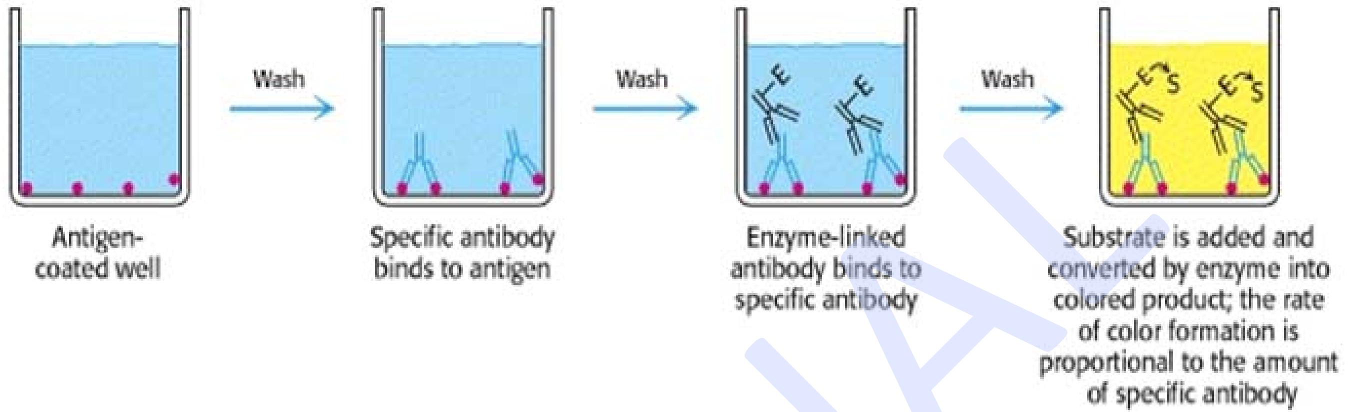
1. Original

J. APPENDICES

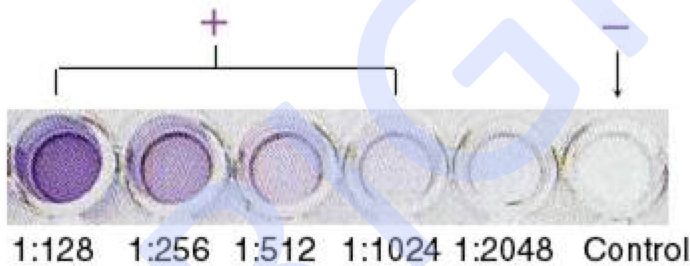
- See following figures for further information

ORIGINAL

General idea of how an indirect ELISA works. In this case we use three antibodies instead of the two shown here.



What you can expect to see in a positive ELISA plate. Note the color lessens as dilutions increase and there is less specific antibody in each successive well. Your negative control well should be colorless.



ORIGINAL

Example of plate layout, readings and findings.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	chall3 4:1 800	chall3 4:1 800	chall3 5:1 800	chall3 5:1 800	chall6 5:1 800	chall6 5:1 800	chall6 6:1 800	chall6 6:1 800	chall6 7:1 800	chall6 7:1 800	chall6 8:1 800	chall6 8:1 800	Well ID Conc/ Dil Name
B	chall3 4:2 1600	chall3 4:2 1600	chall3 5:2 1600	chall3 5:2 1600	chall6 5:2 1600	chall6 5:2 1600	chall6 6:2 1600	chall6 6:2 1600	chall6 7:2 1600	chall6 7:2 1600	chall6 8:2 1600	chall6 8:2 1600	Well ID Conc/ Dil Name
C	chall3 4:3 3200	chall3 4:3 3200	chall3 5:3 3200	chall3 5:3 3200	chall6 5:3 3200	chall6 5:3 3200	chall6 6:3 3200	chall6 6:3 3200	chall6 7:3 3200	chall6 7:3 3200	chall6 8:3 3200	chall6 8:3 3200	Well ID Conc/ Dil Name
D	chall3 4:4 6400	chall3 4:4 6400	chall3 5:4 6400	chall3 5:4 6400	chall6 5:4 6400	chall6 5:4 6400	chall6 6:4 6400	chall6 6:4 6400	chall6 7:4 6400	chall6 7:4 6400	chall6 8:4 6400	chall6 8:4 6400	Well ID Conc/ Dil Name
E	chall3 4:5 12800	chall3 4:5 12800	chall3 5:5 12800	chall3 5:5 12800	chall6 5:5 12800	chall6 5:5 12800	chall6 6:5 12800	chall6 6:5 12800	chall6 7:5 12800	chall6 7:5 12800	Warr No Warr	Warr No Warr	Well ID Conc/ Dil Name
F	chall3 4:6 25600	chall3 4:6 25600	chall3 5:6 25600	chall3 5:6 25600	chall6 5:6 25600	chall6 5:6 25600	chall6 6:6 25600	chall6 6:6 25600	chall6 7:6 25600	chall6 7:6 25600	Conj No Conj	Conj No Conj	Well ID Conc/ Dil Name
G	chall3 4:7 51200	chall3 4:7 51200	chall3 5:7 51200	chall3 5:7 51200	chall6 5:7 51200	chall6 5:7 51200	chall6 6:7 51200	chall6 6:7 51200	chall6 7:7 51200	chall6 7:7 51200	Neg Negative	Neg Negative	Well ID Conc/ Dil Name
H	chall3 4:8 1.02E+05	chall3 4:8 1.02E+05	chall3 5:8 1.02E+05	chall3 5:8 1.02E+05	chall6 5:8 1.02E+05	chall6 5:8 1.02E+05	chall6 6:8 1.02E+05	chall6 6:8 1.02E+05	chall6 7:8 1.02E+05	chall6 7:8 1.02E+05	Pos Positive	Pos Positive	Well ID Conc/ Dil Name

ORIGINAL

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.563	0.554	0.289	0.289	0.571	0.542	0.343	0.334	0.572	0.577	0.053	0.056	405
B	0.488	0.493	0.192	0.209	0.49	0.485	0.468	0.408	0.579	0.601	0.052	0.053	405
C	0.406	0.399	0.138	0.131	0.411	0.387	0.482	0.51	0.597	0.559	0.055	0.055	405
D	0.312	0.306	0.093	0.09	0.286	0.284	0.5	0.512	0.485	0.494	0.059	0.055	405
E	0.233	0.225	0.079	0.077	0.196	0.213	0.592	0.53	0.384	0.379	0.062	0.062	405
F	0.165	0.159	0.062	0.062	0.166	0.146	0.478	0.473	0.309	0.268	0.056	0.052	405
G	0.113	0.11	0.056	0.057	0.096	0.096	0.384	0.385	0.186	0.181	0.065	0.066	405
H	0.084	0.088	0.055	0.053	0.074	0.074	0.287	0.27	0.123	0.125	0.3	0.309	405

	1	2	3	4	5	6	7	8	9	10	11	12	
A	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
B	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
C	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
D	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
E	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
F	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
G	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS	POS	NEG	NEG	Symbols (405)
H	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	POS	Symbols (405)

ORIGINAL

F. psychrophilum ELISA

DATE:____________ CASE #:________________ PLATE #:___________

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Flavobacterium psychrophilum*** **ELISA Plate Reading Using Epoch/Gen5**	
SOP #: 419	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) details the process of reading an enzyme linked immunosorbent assay (ELISA)[1] plate for determination of antigen specific trout antibody to *Flavobacterium psychrophilum* from serum or mucus samples using the Epoch Microplate reader with Gen5 software.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIAL REQUIRED

1. Processed ELISA plates
2. Epoch plate reader
3. Gen5 software
4. Excel software
5. ELISA plate template protocol for Gen5

E. PROCEDURES

1. Turn on laptop computer in laboratory and Epoch plate reader and make sure the USB cable connecting the two is plugged in.
2. Enter username "DWRUSER" and password "Pa$$w0rd" when prompted. When asked if you would like to connect to the ZENworks network, select "Cancel."
3. Double click the "Gen5 1.11 " icon on the desktop and go to the area titled “Create New Item”. Click on “Experiment” and then select “ELISA protocol template”.
4. When the program opens, double click on “protocol” and then on “plate layout”
5. Review the plate layout and change as needed to cover the dilution series you have set up on your ELISA plates. Save your plate layout by clicking on “OK”.
6. Click on “Plate” along the top menu bar.
7. Click “Read” and when prompted save the experiment under a chosen name.
8. Wipe the bottom of each ELISA plate until dry with a Kimwipe.
9. The reader will automatically open the plate carrier drawer and Gen5 will ask you to place plate in the plate carrier. Do so and then select “OK”.
10. The reader will automatically read the plate.
11. When the read is complete remove the plate from the carrier and close the carrier drawer by pushing the button under the drawer.
12. On the Gen5 screen you will be in the “matrix” and “data” view section. On the dropdown menu select “layout”. You will see the layout of the current plate. Click on the export button (Excel button) to send the data to an Excel sheet.

13. On the Excel sheet place your cursor below the exported data and click to set your next insertion point.
14. Go back to Gen5 and on the dropdown menu select "405". You will see the readings from the plate on this screen. Export this data to Excel and once again move your cursor to the next insertion point.
15. Go back to Gen5 and on the dropdown menu select "symbols". Here you will see the readings set out in a language format stating positive and negative findings. Export this data as above.
16. Save your Excel file under a selected name.
17. Go back to Gen5 and click on "File" and save the experiment.
18. Close the Gen5 Program and turn off the spectrophotometer and laptop.

F. QUALITY CONTROL

- Read ELISA plates within 15 minutes of completion of the assay.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 418, *Flavobacterium psychrophilum* Enzyme Linked Immunsorbent Assay
2. Epoch user manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

Example of plate layout, readings and findings.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	chall3 4:1 800	chall3 4:1 800	chall3 5:1 800	chall3 5:1 800	chall6 5:1 800	chall6 5:1 800	chall6 6:1 800	chall6 6:1 800	chall6 7:1 800	chall6 7:1 800	chall6 8:1 800	chall6 8:1 800	Well ID Conc/ Dil Name
B	chall3 4:2 1600	chall3 4:2 1600	chall3 5:2 1600	chall3 5:2 1600	chall6 5:2 1600	chall6 5:2 1600	chall6 6:2 1600	chall6 6:2 1600	chall6 7:2 1600	chall6 7:2 1600	chall6 8:2 1600	chall6 8:2 1600	Well ID Conc/ Dil Name
C	chall3 4:3 3200	chall3 4:3 3200	chall3 5:3 3200	chall3 5:3 3200	chall6 5:3 3200	chall6 5:3 3200	chall6 6:3 3200	chall6 6:3 3200	chall6 7:3 3200	chall6 7:3 3200	chall6 8:3 3200	chall6 8:3 3200	Well ID Conc/ Dil Name
D	chall3 4:4 6400	chall3 4:4 6400	chall3 5:4 6400	chall3 5:4 6400	chall6 5:4 6400	chall6 5:4 6400	chall6 6:4 6400	chall6 6:4 6400	chall6 7:4 6400	chall6 7:4 6400	chall6 8:4 6400	chall6 8:4 6400	Well ID Conc/ Dil Name
E	chall3 4:5 12800	chall3 4:5 12800	chall3 5:5 12800	chall3 5:5 12800	chall6 5:5 12800	chall6 5:5 12800	chall6 6:5 12800	chall6 6:5 12800	chall6 7:5 12800	chall6 7:5 12800	Warr No Warr	Warr No Warr	Well ID Conc/ Dil Name
F	chall3 4:6 25600	chall3 4:6 25600	chall3 5:6 25600	chall3 5:6 25600	chall6 5:6 25600	chall6 5:6 25600	chall6 6:6 25600	chall6 6:6 25600	chall6 7:6 25600	chall6 7:6 25600	Conj No Conj	Conj No Conj	Well ID Conc/ Dil Name
G	chall3 4:7 51200	chall3 4:7 51200	chall3 5:7 51200	chall3 5:7 51200	chall6 5:7 51200	chall6 5:7 51200	chall6 6:7 51200	chall6 6:7 51200	chall6 7:7 51200	chall6 7:7 51200	Neg Negative	Neg Negative	Well ID Conc/ Dil Name
H	chall3 4:8 1.02E +05	chall3 4:8 1.02E +05	chall3 5:8 1.02E +05	chall3 5:8 1.02E +05	chall6 5:8 1.02E +05	chall6 5:8 1.02E +05	chall6 6:8 1.02E +05	chall6 6:8 1.02E +05	chall6 7:8 1.02E +05	chall6 7:8 1.02E +05	Pos Positive	Pos Positive	Well ID Conc/ Dil Name

ORIGINAL

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.563	0.554	0.289	0.289	0.571	0.542	0.343	0.334	0.572	0.577	0.053	0.056	405
B	0.488	0.493	0.192	0.209	0.49	0.485	0.468	0.408	0.579	0.601	0.052	0.053	405
C	0.406	0.399	0.138	0.131	0.411	0.387	0.482	0.51	0.597	0.559	0.055	0.055	405
D	0.312	0.306	0.093	0.09	0.286	0.284	0.5	0.512	0.485	0.494	0.059	0.055	405
E	0.233	0.225	0.079	0.077	0.196	0.213	0.592	0.53	0.384	0.379	0.062	0.062	405
F	0.165	0.159	0.062	0.062	0.166	0.146	0.478	0.473	0.309	0.268	0.056	0.052	405
G	0.113	0.11	0.056	0.057	0.096	0.096	0.384	0.385	0.186	0.181	0.065	0.066	405
H	0.084	0.088	0.055	0.053	0.074	0.074	0.287	0.27	0.123	0.125	0.3	0.309	405

	1	2	3	4	5	6	7	8	9	10	11	12	
A	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
B	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
C	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
D	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
E	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
F	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	Symbols (405)
G	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	POS	POS	NEG	NEG	Symbols (405)
H	NEG	NEG	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	POS	Symbols (405)

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Probiotic Growth and Feed Preparation**	
SOP #: 420	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Bacteriology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to grow the probiotic C6-6 or C6-8 (provided by Ken Cain, University of Idaho) and topcoat fish feed for control of *Flavobacterium psychrophilum.*

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working with all bacterial cultures.
- Treat all bacterial cultures as if they were pathogenic.
- Use aseptic technique when working with live bacterial cultures.
- Disinfect all surfaces before and after working with any bacterial cultures[2]. Dispose of wastes[3] and sharps properly[4].
- When working with large amounts of this bacterium and/or top coating feed: it is advisable to wear gloves, protective clothing that can be disposed of/washed and a respirator. Do not handle this bacterium if you have open sores or cuts or if you are immunocompromised.
- When feeding fish with this probiotic: This probiotic is a beneficial bacterium, isolated from healthy fish. Risk of infection from this organism is very low, however; it is prudent to avoid accidental inoculation and ingestion. You should avoid handling if you have open cuts or are taking any medications that suppress your immune system. Please wear gloves when handling the probiotic. Wash your hands after handling the feed. Apply bleach or 70% alcohol to spills.

D. EQUIPMENT AND MATERIALS REQUIRED

1. TSB[5]
2. TSA plates[6]
3. Parafilm
4. Probiotic of choice
5. Sterile culturing loops
6. 15 mL or 50 mL conical tubes
7. Centrifuge
8. Fish oil
9. Incubator
10. Gram stain slides
11. Gram stain reagents
12. API strips and associated reagents (See manufacturer's suggestions.)
13. Vortexer
14. Fish feed

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15. Containers to hold fish feed
16. Mixer
17. Gloves and other safety gear

E. PROCEDURES

1. Streak fresh TSA plates with C6-6 or C6-8 culture so as to obtain distinct separate colonies for isolation and testing.
   - Incubate the culture at 15ºC (this probiotic grows at room temperature as well) to produce a fresh log phase growth culture (approximately 24-48 hours of growth).
2. Inoculate the required amount of fresh TSB for production of appropriate amounts of bacteria.
   - Place inoculated tubes or flasks at desired temperature (15ºC or room temperature) for approximately 48 to 72 hours (this probiotic grows at room temperature and 100 L fermentations were run at room temperature).
3. After incubation, centrifuge at 2600-3000 rpm for 15-20 minutes and discard supernatant.
4. Prepare needed volume of bacteria:
   - Using the equation determined by David Burbank based on drop plate method for colony counting and enumeration:

     15 mL of bacteria in broth (spun down and supernatant discarded) should be suspended in 30 mL of fish oil and then that 30 mL will topcoat 300g feed.
   - It was found that the amount of oil needed to be adjusted based on the types and sizes of feed.
     - Automatic feeders may get clogged if too much oil is used.
     - As feed sizes increase, the feed will generally hold or need less oil.
     - The amount of bacteria should remain as it was calculated and only the volume of fish oil used should be adjusted.
     - A few examples of troubleshooting:
       - Starter: 10 pounds feed would have required 453 mL fish oil based on the above calculation, however 300 mL of oil applied to the starter feed clogged the automatic feeders. When we used 200 mL oil it did not clog the feeders.
       - #1 feed: 14 pounds would have required 635 mL of oil - cut this back to 300 mL.
       - #2 feed: 45 pounds would have required 2041.2 mL of oil- cut this back to 1200 mL
       - #2 feed: 100 pounds would have required 4537 mL oil- cut this back to 3500 mL.
5. Mixing feed:
   - Mix fish oil/probiotic solution thoroughly.
     - For large trials a cement mixer may be required. When using a cement mixer it may be necessary to reach deep into the mixer to distribute oil evenly. Pour oil in slowly as mixer starts turning.
     - Starter feed tends to clump very easily, it is recommended to screen it through a small soil sieve to help break up the clumps.
6. Store feed either in refrigerator (4°C) for up to a week or frozen (-20°C) for up to 30 days.

F. QUALITY CONTROL

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- Gram stain[6] and verify culture using API test strips[7,8].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 209, Disposal of Hazardous Wastes
4. SOP# 205, Handling and Disposal of Sharps
5. SOP# 836, TSB Preparation
6. SOP# 837, TSA Preparation
7. SOP# 302, General Necropsy and Sample Collection for Inspections
8. SOP# 303, Field Collection of Bacteriology Samples
9. SOP# 404, Gram Staining
10. SOP# 406, API 20E
11. SOP# 407, API 20NE
12. Assessing Probiotic Use for the Control of *Flavobacterium psychrophilum* in Rainbow Trout (*Oncorhynchus mykiss*). A Thesis by David Ryan Burbank, June 2011, University of Idaho.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

Parasitology 500

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Head Defleshing Procedure in Preparation for Pepsin-Trypsin Digestion (PTD)**	
SOP #: 501	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the process in which skin and muscle tissue are removed from a fish head and remaining bone and cartilage are retained for Pepsin Trypsin Digest analysis (PTD).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure safety procedures[1] when working with reagents and sharp instruments[2] used in this protocol. See associated SOPs for specific safety measures when preparing and using each of the reagents[3] utilized in this SOP.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Whirl-Pak bags
2. Autoclave bags, clear 2.0 pp VWR 95042-563 10x15
3. Fish heads collected from inspection kept cold (4°C) but not frozen
4. Falcon 50 mL tubes Cat # 21008-951 or equivalent
5. Beakers and flasks
6. Tube racks
7. Cutting board
8. Scalpel and blade # 22
9. Butcher paper
10. Sterile forceps
11. Scalpel blades and handles
12. Precision water bath
13. 1 Gallon Ziploc bags
14. Disposable paper paint filters

E. PROCEDURES

1. Sample Preparation:
   - Prepare lab bench with butcher paper, cutting board, dissecting tools, scalpels, scalpel blades and appropriate waste containers (i.e., autoclave bags and bench top waste containers.)
   - Retrieve fresh heads from 4°C (heads to be processed for PTD should NEVER be frozen at -20°C).
   - Pour whole fish heads into a new watertight Ziploc bag, add water, agitate to rinse blood from samples and remove excess organs (liver, heart, etc.).
   - Prepare and label a set of 50 mL conical tubes (one for each individual fish sample).
   - Prepare and label a set of Whirl-paks (5 fish/pool).

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- Work in pools of 5 fish. Split the head samples longitudinally using a scalpel blade and place one half in the labeled (5/pool) Whirl-pak and the other half into the corresponding labeled 50 mL conical tube. Repeat until all samples have been split and placed accordingly.
  - If fish are too small to be appropriately split, the whole head can be processed.
  - Alternatively, if the fish head is too large a large butcher knife and mallet can be used to split the sample or a core or wedge sample can be taken.
  - Wedge samples are collected by cutting a triangle-shaped section from the fish head. The cut should be made on top (dorsal) surface posterior to the eyes and extend to the top of the mouth. The top (dorsal) portion of the wedge should measure ~1.5 cm.
  - Core samples are taken by inserting a biopsy or boring tool at least 19 mm diameter into the dorsal surface of the head just posterior to the eyes and forcing it ventrally until it penetrates into the mouth.
- Place all filled Whirl-paks in a large plastic bag that has been labeled with location, date, sample time and case number. Archive these samples in the sample freezer near the West Lab.
- Fill each 50 mL conical tube that contains a half head sample with approximately 40-45 mL of tap water. Place tubes in large plastic bag (two bags needed for a case of 60 fish).
- Place bags (and use binder clips to secure) in water/shaker bath and set to 45°C. Cook samples until eyes are opaque, checking periodically to prevent overcooking. Fully cooked heads can be stored at 4°C until ready for next processing step.

Small head sample cooked until eyes are opaque

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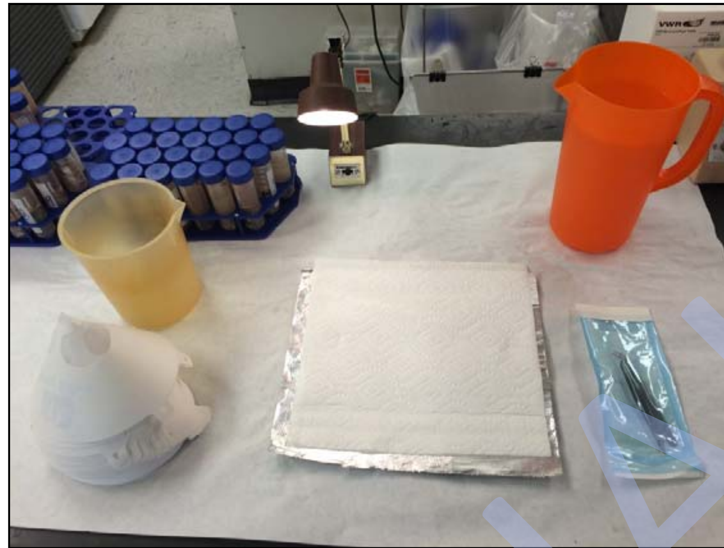
2. Defleshing Procedure:
   - Cover counter top with a large sheet of butcher paper. Place folded paper towel on top of a sheet of tinfoil as shown below.
   - Process heads individually by first decanting water from each sample through a paint filter and into a waste bucket. The filter will retain the head in the event it is accidentally poured out of the cooking container.



Defleshing workspace

- Remove fish head from container. Place on paper towel and remove eyes, skin and any large pieces of flesh using a pair of forceps. Place this material somewhere on the paper towel to be discarded. Take care to retain all hard material which will include cartilage and bone. Place this hard material into a 50 mL conical tube, add tap water to the tube until approximately ½ full.
- Tightly cap and vigorously shake to remove excess soft tissue. Carefully decant water through the filter while retaining cartilage and bone.
- Add additional tap water and repeat. Continue this procedure until remaining cartilage/bone appears clean and soft tissue has been decanted through rinsing. This process will minimize the amount of pigment present in the sample, which can interfere with myxopsore examination following the PTD procedure.
- Carefully (using forceps) examine decanted material present in the filter for cartilage/bone. Grasp any pieces and add them back to the tube.

50 mL tube containing cleaned cartilage and bone

- Ensure the tube is appropriately labeled and capped. Place it in a rack. Defleshed samples may be refrigerated overnight. Samples are now ready for pepsin digestion.

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F. QUALITY CONTROL

- If a case contains multiple lots, these groups should be separated and processed individually.
- Only one lot may be open at a time at each workstation.
- Multiple lots may be cooked in the same water bath if they are from the same case and kept separate while cooking.
- If enough water baths are available, treat different lots as separate cases. **DO NOT** mix cases in the water bath or at workstations.
- Disinfect all surfaces and equipment with a 10% bleach solution after use.
- Clean, drain, and dry water baths[5] after use.

G. INTERPRETATION

See Pepsin-Trypsin Digestion (PTD) For Recovery of *Myxobolus cerebralis* Spores[4] for further processing steps.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 209, Disposal of Hazardous Waste
4. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *Myxobolus cerebralis* Spores
5. SOP# 506, Decontamination of Equipment/Tools After *M. cerebralis* Processing
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HIISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Pepsin-Trypsin Digestion (PTD) for Recovery of *Myxobolus cerebralis* Spores**	
SOP #: 502	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to extract *Myxobolus cerebralis* spores from fresh cartilage using the Pepsin-Trypsin Digest (PTD). Pepsin is a digestive enzyme used to dissolve skeletal material and cartilage, release encysted myxospores and make them available for concentration. The rate of fish bone digestion is related to the ratio of pepsin to bone. Following the pepsin digestion, the sample is further digested by another enzyme, trypsin, which breaks down proteins.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure personal protective procedures[1] when working with reagents used in this protocol. See associated SOPs for specific safety measures[2] when preparing and using each of the reagents[3] utilized in this SOP.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Autoclave bags, clear 2.0 pp 10x15 (VWR 95042-563 or equivalent)
2. Scale
3. Tube racks
4. Butcher paper
5. Water baths
6. Beakers and flasks
7. Centrifuge
8. Falcon 50 mL tubes (Cat # 21008-951 or equivalent)
9. 1 gallon plastic bags
10. Disposable paper paint filters
11. pH indicator strips (Fisher Cat# M1095020001 or equivalent)
12. 0.5% Pepsin Solution[4]
13. Trypsin Solution[5]
14. 10% Bovine Serum Albumin[6]
15. Rinaldini's Solution[7]
16. 53% Sucrose Solution[8]
17. 0.5N Sodium Hydroxide[9]

E. PROCEDURES

1. Pepsin Digestion
   - Prepare lab bench with butcher paper and appropriate waste containers (i.e., autoclave bags and bench top waste containers).

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- Turn on water/shaker baths. Set temperatures to 37°-40°C. Set shaking action to 70-130.
- Prepare 0.5% Pepsin Solution. Invert several times to ensure an adequate mixing, particularly if the solution has been prepared ahead of time and stored at 4°C until use. Swirl several times in 6 L Erlenmeyer flask before aliquoting to smaller dedicated container for each case. This will help reduce the risk of contamination of the larger volume of pepsin solution.
- Start with defleshed head samples[10]. Each defleshed head sample will be in a labeled 50 mL tube.
- Fill each 50 mL tube (containing the defleshed head sample) with Pepsin Solution to approximately the 40 mL mark. Tighten screw cap and repeat until all 60 samples are complete.
- Place all 50 mL tubes into a plastic bag. Orient the samples so that the shaking motion of the water bath facilitates optimal motion of the samples.
- Place plastic bags into water bath. Use binder clips to attach bags to the shaking platform to facilitate optimal motion of the samples.
- Allow samples to shake until bones and cartilage have digested and resemble "fine sand". Samples need to be monitored daily for exhaustion of pepsin solution, as indicated by an increase in pH to approximately 4.0. The rate at which pepsin will exhaust is dependent upon the amount of bone present in the samples. The larger samples will cause a quicker increase in the pH and require more frequent reagent changes.
- To check the integrity of the pepsin solution, remove 1 50 mL tube from each bag and insert a new pH strip. Check the color labels on manufacture's box to read pH of each sample. See following picture.

- If solution is at a pH of 4.0 or higher, the reagent is exhausted and must be changed. To do so, centrifuge samples at 2050 rpm for 10 minutes. Carefully decant supernatant into waste bucket.

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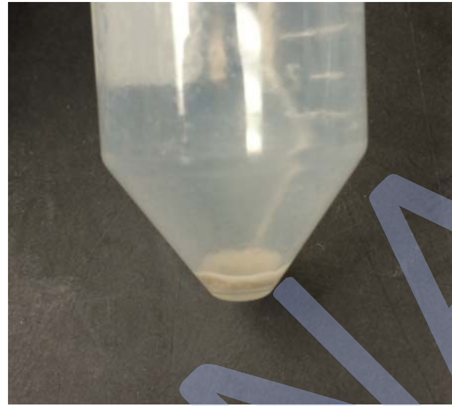
- Repeat at step 5 until all bones and cartilage have digested completely and resemble "fine sand", checking pH daily, and refreshing pepsin as needed.
- When individual bone/bone fragments are no longer recognizable and just a pellet remains after centrifugation, the sample is completely digested and ready for the Trypsin portion of the PTD. See following picture.



- When samples are completely digested and resemble above photo, carefully decant supernatant into waste bucket. Samples may be stored at 4°C like this until all samples are ready to proceed to trypsin digestion.
- Add generous amount of bleach to waste bucket (approximately 25% of volume) and allow to sit for approximately 10 minutes and dispose of down the drain. Rinse sink thoroughly to remove waste and fat.
- Decontaminate tools and equipment[11].

2. Trypsin Digestion
   - Prepare lab bench with butcher paper and appropriate waste containers (i.e., autoclave bags and bench top waste containers).
   - Retrieve completely pepsin-digested samples.
   - Retrieve and/or prepare 0.5% Trypsin Solution.
   - Samples will be combined 5 per pool. Pour 20 mL of 0.5% trypsin solution to the first sample of the pool (i.e., sample #1). Vortex, invert and/or use a new disposable transfer pipette to thoroughly mix and dislodge the pellet from the bottom of the tube.
   - Using a transfer pipette, transfer the homogenous mixture to the next sample for the pool (i.e., sample #2). Vortex, invert and/or use a new disposable transfer pipette to thoroughly mix and dislodge the pellet from the bottom of the tube. Repeat this process until the last sample of the pool (i.e., sample #5). Continue for all samples. There should be 12 total pools for a case of 60 fish.
   - Adjust the pH of each sample to approximately 8.0-8.2 (should be a pink color similar to the starting color of the trypsin solution). Check the pH using the pH indicator strips (or by comparing the color to the starting color of the trypsin solution) and adjust using 0.5N NaOH and a transfer pipette. Add NaOH carefully drop by drop so as not to exceed a pH of 8.4 as this will cause morphological damage to the myxospre polar capsules. If several drops of NaOH are required, you can use 1.0N NaOH.
   - Samples must remain in trypsin solution for 30 minutes. During this digestion, agitate/invert samples several times and readjust pH as necessary. Can place tubes in

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plastic bag and place in shaking water bath if necessary to facilitate shaking during digestion.
- After the 30 minute digestion period, inactivate/halt trypsin digestion by adding 5 mL 10% bovine serum albumin to each pooled sample. Invert/mix. Solution should turn back to a yellowish color.
- Ensure each lid is secure and centrifuge at 2050 rpm for 10 minutes. Decant supernatant into waste bucket. Sample is now ready for glucose/sucrose filtration[12].
- Add generous amount of bleach to waste bucket (approximately 25% of volume) and allow to sit for approximately 10 minutes and dispose of down the drain. Rinse sink thoroughly to remove waste and fat.
- Decontaminate tools and equipment for complete clean-up.

F. QUALITY CONTROL

- To ensure no cross-contamination between cases and lots, work on a single case at a time and decontaminate between cases.

G. INTERPRETATION

See Glucose/Sucrose Filtration for the Concentration of *M. cerebralis* Spores and Staining and Reading PTD Slides for Further Analyses[13].

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 209, Disposal of Hazardous Wastes
4. SOP# 825, Pepsin Solution
5. SOP# 835, Trypsin Solutions
6. SOP# 802, 10% Bovine Serum Albumin
7. SOP# 282, Rinalidini's Solution
8. SOP# 831, 53% Sucrose Solution
9. SOP# 830, 0.5N Sodium Hydroxide (NaOH) Solution
10. SOP# 505, Processing Head Samples in Preparation for DNA Extraction
11. SOP# 506, Decontamination After *Myxobolus cerebralis* Processing
12. SOP# 503, Glucose/Sucrose Filtration for *Myxobolus cerebralis* Spore Concentration
13. SOP# 504, Staining and Reading Slides for the Detection of *Myxobolus cerebralis* Spores
14. SOP# 807, 55% Dextrose Solution Preparation
15. SOP# 805, Carbol Fushsin Stain Preparation
16. SOP# 816, Methyl Blue Stain Preparation
17. SOP# 821, Phosphate Buffered Saline (PBS)
18. SOP# 819, Myxospore Resuspension Media
19. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

ORIGINAL

J. APPENDICES

- N/A

ORIGINAL

State of Utah

Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Glucose/Sucrose Filtration for *Myxobolus cerebralis* Spore Concentration**	
SOP #: 503	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure for the concentration of *M. cerebralis* spores after a pepsin-trypsin digestion of fresh tissue in preparation for making and reading slides to interpret the status of *M. cerebralis* infection.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1].
- Use caution when disposing of infectious materials[2] and hazardous wastes[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Autoclave bags, clear 2.0 pp 10x15 (VWR 95042-563 or equivalent)
2. Butcher paper
3. 15 mL tubes
4. 53% Sucrose Solution[4] OR 55% Dextrose Solution[5]
5. Centrifuge
6. Sterile PBS[6]
7. Ethanol (EtOH)
8. dH_2O
9. Bench top liquid waste container
10. Disposable transfer pipettes
11. Samples that have been thoroughly pepsin and trypsin digested

E. PROCEDURES

1. Prepare lab bench with butcher paper and appropriate waste containers (i.e., autoclave bags and bench top waste containers).
2. Prepare and label a set (12 tubes for a case of 60, 5samples/pool) of 15 mL tubes with 5 mL each of either 53% sucrose solution[4] or 55% dextrose solution.
3. Retrieve samples that have been thoroughly pepsin-trypsin digested and re-suspend each pellet with 1 mL of re-suspension fluid[7]. Vortex and/or use transfer pipette to thoroughly mix and re-suspend.
4. Carefully layer suspension on top of the sucrose column for each sample.
5. Centrifuge at 2050 rpm for 30 minutes.
6. Carefully decant entire supernatant into the waste bucket. Take caution to remove the entire volume of the supernatant, this may be achieved by using a transfer pipette, leaving a pellet.
7. Prepare or retrieve re-suspension media.

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8. Add 500 µL of myxospore resuspension media to each sample, mix. Samples are now ready to be fixed on slides and stained.
9. If slides are not to be stained immediately afterwards, refer to Decontamination of Equipment/Tools After *M. cerebralis* Processing[8].
10. If slides are to be stained immediately following completion of sucrose/glucose filtration, see Staining and Reading Slides for the Detection of *M. cerebralis* Spores[9].

F. QUALITY CONTROL

- Process only one case at a time to keep cross-contamination to a minimum. Use proper clean-up techniques on work space before moving to a second case and when done processing samples. Any positive controls should not be processed during the same time as samples to be analyzed.

G. INTERPRETATION

Stain and read samples.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 209, Disposal of Hazardous Wastes
4. SOP# 831, 53% Sucrose Solution Preparation
5. SOP# 807, 55% Dextrose Solution Preparation
6. SOP# 821, Phosphate Buffered Saline (PBS)
7. SOP# 819, Myxospore Resuspension Media
8. SOP# 506, Decontamination of Equipment/Tools after *M. cerebralis* Processing
9. SOP# 504, Staining and Reading Slides for the Detection of *M. cerebralis* Spores
10. SOP# 501, Head Defleshing Procedure in Preparation for Pepsin-Trypsin Digestion (PTD)
11. SOP# 805, Carbol Fushsin Stain
12. SOP# 816, Methyl Blue Stain
13. SOP# 830, 0.5N Sodium Hydroxide (NaOH) Solution
14. American Fisheries Society Fish Health Section Blue Book

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Staining and Reading Slides for the Detection of *M. cerebralis* Spores**	
SOP #: 504	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/20/2017

A. PURPOSE

This standard operating procedure (SOP) describes the procedure for staining and reading slides for the detection of *M. cerebralis* spores after complete pepsin-trypsin digestion (PTD) of fresh tissues and myxospore concentration by glucose/sucrose filtration.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1].
- Use caution when disposing of infectious materials[2] and hazardous wastes[3].
- Use caution and appropriate PPE when handling reagents[4] used in staining (i.e., carbol fuchsin, methyl blue and xylene).

D. EQUIPMENT AND MATERIALS REQUIRED

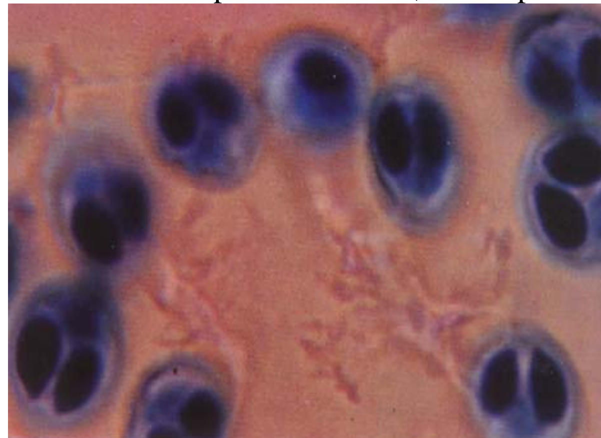
1. Butcher paper
2. 12-spot microscope slides
3. Slide dryer
4. Sterile disposable transfer pipettes
5. Microcentrifuge tubes
6. Myxospore re-suspension media[5]
7. Carbol fuchsin stain[6]
8. Methyl blue stain[7]
9. Decolorizing agent (Fisher Cat# 212528)
10. dH_2O
11. Xylene (or xylene substitute)
12. Permount microscope slide fixative
13. Coverslips
14. Immersion oil
15. Microscope

E. PROCEDURES

1. Slide staining:
    - Prepare lab bench with butcher paper and retrieve pepsin-trypsin digested and sucrose/glucose filtered samples.
    - Using a sterile transfer pipette, place a drop of sample on one well of multi-spot slide. Repeat so you have two replicates from each sample (i.e., two wells on the multi-spot slide will be from the same sample). Be sure to label or note the location of each well and which sample it coordinates with.
    - Repeat step 2 for all samples in the case.

- Place slides on slide dryer for approximately 2 hours or until dry
- Once all sample slides have been placed on slide dryer, retrieve positive control from -20°C, vortex and aliquot 1 mL to a new microcentrifuge tube. Centrifuge at 14,000 rpm for 3 minutes.
- Decant supernatant from positive control sample into waste container and re-suspend pellet in 100 µL of myxospore re-suspension media.
- Repeat step 2 with prepared positive control. Be sure to put positive controls on a separate slide than any other samples. Place positive control slide on slide dryer for approximately 2 hours or until dry.
- Once all slides (sample and control slides) are dried on slide warmer, place them in a stand-up slide rack and place in warming incubator at 54°C overnight.
- Retrieve the slides from the warming incubator and flood each sample slide with carbol fuchsin for 20 minutes, then rinse gently with dH_2O. Process sample slides first before handling positive control slide.
- Decolorize sample slides gently with decolorizing agent for approximately 30 seconds.
- Rinse sample slides with dH_2O.
- Flood each sample slide with methyl blue stain for 2 minutes.
- Decant methyl blue off slide and rinse with dH_2O. Allow slides to air dry.
- Repeat steps 8-12 for positive control slide after all sample slides have been stained.
- After slides have completely dried, coverslips may be permanently mounted to each slide. Dip entire slide in xylene briefly (let drips flow off slide onto paper towel).
- Place one drop of Permount onto slide and place a large coverslip over entire slide. Ensure no air bubbles are trapped under coverslip and use paper towel or Kimwipe to absorb extra fluid.
- Place slides in slide box and next to microscope if not to be read immediately.

2. Slide reading:
    - To ensure staining was successful, proceed with the following steps starting with the positive control slide, and then moving on to the sample slides.
    - Add a drop of immersion oil to each of the wells in the first row of the multi-spot slide (when lens passes through oil from top row, the oil will drag to the second row coating the second row of wells).
    - Observe for the presence of myxospores using 40X oil objective, must observe a minimum of 150 fields per SAMPLE (not per replicate well of sample).
    - If no spores are observable in positive control, then repeat staining.


USFWS

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F. QUALITY CONTROL

- Ensure no cross-contamination between positive control and samples to be tested. Process one lot at a time and thoroughly disinfect all surfaces and equipment with 10% bleach solution after use[8].

G. INTERPRETATION

Myxospores will stain red/pink while surrounding debris and other materials will counterstain blue. Myxospores will be 8 to 10 µm, rounded, with two polar capsules. A single myxospore of appropriate size is sufficient to declare a PRESUMPTIVE positive. Other myxobolid species of salmon have similar morphologies that may be confused with *M. cerebralis*; see photos below and refer to positive control for confirmation of morphology.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 208, Disposal of Infectious Materials
3. SOP# 209, Disposal of Infectious Wastes
4. SOP# 206, Handling of Hazardous Materials and Chemical Inventory
5. SOP# 819, Myxospore Re-suspension Media
6. SOP# 805, Carbol Fushin Stain
7. SOP# 816, Methyl Blue Stain Preparation
8. SOP# 506, Decontamination of Equipment/Tools After *M. cerebralis* Processing
9. SOP# 501, Head Defleshing Procedure in Preparation for Pepsin-Trypsin Digestion (PTD)
10. SOP# 504, Staining and Reading Slides for the Detection of *M. cerebralis* Spores
11. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Processing Head Samples in Preparation for DNA Extraction**	
SOP #: 505	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the sample processing procedure of head tissues for the preparation of DNA extraction prior to PCR for the detection of *Myxobolus cerebralis* spores. These steps have been optimized to ensure an appropriate sized sample and to give an adequate amount and concentration of DNA.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Always utilize personal protective equipment[1] and use caution with hazardous wastes[2].
- Ensure safety procedures when working with sharp objects such as scalpel blades[3].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Butcher paper
2. Biohazard waste container
3. Whirl-paks
4. Cutting board
5. Scalpel handle
6. Scalpel blade(s)
7. Sterile forceps
8. Large Ziploc bags
9. Biopsy punches (6 mm)
10. Sterile wooden sticks
11. 2 mL Eppendorf microcentrifuge tubes
12. Microcentrifuge tube racks
13. Paper towel
14. Aluminum foil

E. PROCEDURES

1. Retrieve frozen head samples[4] from 4°C and place in “defrosting” drawer black media fridge. Samples should thaw until they are no longer completely frozen solid but are still somewhat frozen as this will make it easy to split samples with scalpel.
2. Line lab bench with butcher paper, cutting board and prepare a biohazard waste bag.
3. Label Whirl-paks for 5 samples/pool with case location and sample number (i.e., 1-5).
4. Label large Ziploc bag with case location and date head samples were processed.
5. Prepare and label enough microcentrifuge tubes for the entire case (individually labeled with sample number).
6. Work in pools of 5 heads. Split heads sagitally using scalpel and place one section of each head into appropriate Whirl-pak.

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7. Use biopsy punch to remove a small core of tissue (use a new punch for each pool of 5). Biospy the ventral calvarium, poster to the optic lobe and including the majority of the auditory capsule. Use a sterile wooden stick (new stick for each pool of 5) to dislodge head core from punch into labeled microcentrifuge tube.
8. Repeat until all samples have been biopsied.
9. If necessary, remove skin and muscle so that no more than 120 mg of tissue will be used for the extraction process (weigh several individual samples to obtain an average weight).
10. Add 180 µL of ATL Buffer (Qiagen® DNeasy® Extraction Kit) and 20 µL proteinase K per 25 mg of tissue (i.e., 120 mg tissue = 900 µL of ATL Buffer + 100 µL proteinase K). Vortex.
11. Invert microcentrifuge tube racks so tubes are horizontal and wrap racks together using aluminum foil. This will ensure the lysis buffer will continuously move across tissue during digestion.
12. Place inverted and wrapped racks in incubator on shaking platform and set to 56°C.
13. Tissues need to digest for at least approximately 24 hours. Tissues can remain in incubator for multiple days if necessary.
14. After proteinase K/ATL digestion, samples can be stored for up to 6 months at ambient temperature without any reduction in DNA quality.
15. Follow Decontamination of Equipment/Tools After *M. cerebralis* Processing[5] for complete clean-up. Use bleach and/or DNAway to thoroughly disinfect all working areas and materials used.

F. QUALITY CONTROL

- Process only one case at a time to ensure no cross contamination between cases. Remove biohazard waste when done processing and autoclave as soon as possible.

G. INTERPRETATION

See DNA Extraction Method from Head Tissues for *M.* cerebralis PCR[6] and *Myxobolus cerebralis* Nested Polymerase Chain Reaction[7].

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 209, Disposal of Hazardous Wastes
3. SOP# 205, Handling and Disposal of Sharps
4. SOP# 310, Field Collection of *Myxobolus cerebralis* Samples
5. SOP# 506, Decontamination of Equipment/Tools After *M. cerebralis* Processing
6. SOP#703 , DNA Extraction Method from Head Tissues for *M.* cerebralis PCR
7. SOP# 707, Myxobolus cerebralis Nested Polymerase Chain Reaction
8. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
9. SOP#708, *Myxobolus cerebralis* Polymerase Chain Reaction; Single Round

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Decontamination After *Myxobolus cerebralis* Processing**	
SOP #: 506	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the decontamination protocols to ensure proper clean up after processing or working with tissues to be analyzed for the presence of *M. cerebralis* spores. These methods are to ensure all materials are non-infective when disposed of properly in the laboratory and that benches and equipment are thoroughly decontaminated. When completed with any sample processing in which *M. cerebralis* could be suspected or detected, appropriate decontamination procedures must be followed. It's important to treat all samples as if they could be positive, this will ensure adequate aseptic technique when processing and will enforce the importance of proper decontamination of all surfaces after processing.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure proper ventilation when handling bleach solution. Bleach solution is corrosive, keep out of eyes and wear appropriate personal protective equipment[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Freshly prepared 10% bleach solution
2. Paper towels
3. Biohazard waste bags
4. Autoclave
5. Small plastic soaking tub

E. PROCEDURES

1. Remove all tools and equipment used.
   - All dissecting tools must be placed in small bleach water solution near sink to soak before being washed.
   - Cutting boards, tube racks and plastic water pitchers must be placed in large bleach water bucket near lab door; these materials must remain in bleach water for at least 30 minutes before being scrubbed and rinsed. If any materials were used as waste containers during defleshing of heads, they must be placed in small plastic bleach bucket in sink (and NOT in the large bleach bucket on the ground near the door—these materials are coated in fat).
   - Butcher paper to be placed in large biohazard waste bag.
2. Disinfect lab bench/large equipment used.
   - Liberally squirt lab bench with freshly prepared bleach solution and use paper towel to wipe. Let air dry.

- If decontaminating after working with DNA, use DNAway after using bleach. Make sure to decontaminate any and all equipment used (i.e., centrifuge, pipettes, etc).
- Spray 70% isopropyl alcohol on lab bench after disinfected with bleach and use paper towel to wipe. Let air dry

3. Biohazard removal:
- Remove biohazard waste bag, compress and loosely tie shut.
- Place in large autoclave bin and slide into autoclave.
- Fill autoclave water reservoir, seal and autoclave for at least 15 minutes. Replace waste container with new bag.

F. QUALITY CONTROL

- Multiple times throughout the year quality control procedures are performed on common laboratory equipment and spaces to ensure proper decontamination[2].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 506, Decontamination of Equipment/Tools After M. cerebralis Processing
3. SOP# 501, Head Defleshing Procedure in Preparation for Pepsin-Trypsin Digestion (PTD)
4. SOP# 502, Pepsin Trypsin Digestion for the Recovery of Myxobolus cerebralis Spores
5. SOP# 503, Glucose/Sucrose Filtration For *M. cerebralis* Spore Concentration
6. SOP# 504, Staining And Reading Slides For The Detection Of M. cerebralis Spores
7. SOP# 505, Processing Head Samples in Preparation for DNA Extraction
8. SOP# 703, DNA Extraction Method from Head Tissues for Myxobolus cerebralis PCR
9. SOP# 707, Myxobolus cerebralis Nested Polymerase Chain Reaction
10. SOP# 708, Myxobolus cerebralis Polymerase Chain Reaction Single Round

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah

Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: *Bothriocephalus acheilognathi* Sample Processing and Identification**	
SOP #: 507	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: M. Hansen & D. Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods for screening fresh gastrointestinal tract tissue for the presence of adult Asian tapeworm (*Bothriocephalus acheilognathi*).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure personal protective equipment[1] and safety procedures when working with sharp instruments[2] used in this protocol.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Scalpel and blades
2. Clean Petri dishes
3. Forceps
4. Phosphate buffered saline[3] (PBS)
5. Dissecting microscope

E. PROCEDURES

1. Perform a general necropsy[4] on fish to be inspected for the presence of Asian tapeworm.
2. Collect fresh gastrointestinal tract tissue samples[5].
3. Line up section(s) of intestine on the top of the small side of a Petri dish. Tissue from smaller fish may be processed up to 5 at a time on a single Petri dish.
4. Moisten the large side of the Petri dish with PBS and place the small side (including tissue) on top and press the tissue flat. Intestine is now easily viewed under a dissecting microscope.

ORIGINAL

F. QUALITY CONTROL

- Handle all samples with gloves and take care to avoid cross contamination between samples and especially between cases.
- Samples must be processed and examined in a timely manner so live parasites can be visualized.

G. INTERPRETATION

Examine fresh tissues for the presence of Asian tapeworm. Adult tapeworms will have a very distinct scolex shaped like an arrowhead. The body will consist of flattened proglottids. Adults can grow to a length of 3.5 cm to 8 cm with widths up to 4 mm. If adult tapeworms, larvae or eggs are detected, document the sample as positive on the inspection form.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 821, Phosphate Buffered Saline, PBS
4. SOP# 302, General Necropsy and Sample Collection for Inspections
5. SOP# 309, Field Collection of *Bothriocephalus acheilognathi* Samples
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Tetracapsuloides bryosalmonae* Sample Processing and Identification**	
SOP #: 508	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods for screening kidney and/or spleen tissue imprints for the presence of *Tetracapsuloides bryosalmonae* parasites, the causative agent of Proliferative Kidney Disease (PKD).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1] when handling hazardous reagents such as methanol and Giemsa stains.
- Ensure safety procedures when working with sharp instruments[2] used in this protocol.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Microscope
2. Immersion oil
3. Giemsa stain
4. Methanol
5. dH_2O
6. Coplin jars

E. PROCEDURES

1. Collect samples[3] and prepare tissue imprints[4], allow slides to completely air dry.
2. Preparing Giemsa stain:
   - Dilute Giemsa stain[5] 1:20 with dH_2O in Coplin jar.
3. Staining of tissue imprints:
   - Fix slides in 100% methanol for 20 minutes.
   - Completely air dry slides.
   - Submerge slides in Coplin jar containing diluted Giemsa stain for 15-60 minutes (depending on intensity desired).
   - Rinse in dH_2O.
   - Completely air dry slides.
4. Reading slides:
   - View stained slides under microscope.
   - Wet mounts may also be used for presumptive diagnosis, in which the PKD parasite occurs within the kidney intersititium.

F. QUALITY CONTROL

- Handle all samples with gloves and take care to avoid cross contamination between samples and especially between cases.

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- Samples must be processed and examined in a timely manner to ensure sample integrity.

G. INTERPRETATION

PKD parasites are round, 10 to 20 µm in diameter and have a light staining, vacuolated cytoplasm.

One to seven secondary (daughter) cells are found within the cytoplasm of the primary (mother) cell. Intensely stained inflammatory cells often are attached to and surround the parasite. PKD organisms can be differentiated from host cells by the presence of a distinctive cell membrane and small nuclei.

Confirmatory diagnosis must be completed by histological examination of tissue sections stained with hematoxylin and eosin. The parasite is amoeboid, 5-20 µm in diameter and has a foamy, eosinophilic cytoplasm. The primary cell contains 1-3 nuclei.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 307, Field Collection of *Tetracapsuloides bryosalmonae* Samples
4. SOP# 402, Staining of Smears and Imprints
5. SOP# 812, Giemsa Stain
6. SOP# 302, General Necropsy and Sample Collection for Inspections
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: *Ceratonova shasta* Sample Processing and Identification**	
SOP #: 509	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Parasitology	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes the appropriate methods for screening fresh gastrointestinal tract tissue for the presence of *Ceratonova shasta*.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure personal protective equipment[1] and safety procedures when working with sharp instruments[2] used in this protocol.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Scalpel and blades
2. Clean petri dishes
3. Forceps
4. Phosphate buffered saline (PBS)
5. Light microscope
6. Immersion oil

E. PROCEDURES

1. Perform a general necropsy[3] on fish to be inspected for the presence of *Ceratonova shasta*.
2. Collect fresh gastrointestinal tract tissue[4] samples.
3. Scrape the posterior 1/3 of the intestinal mucosa and mix in a drop of water or PBS[5] on the microscope slide.
4. Scan wet mount under 200 to 440x magnification.

F. QUALITY CONTROL

- Handle all samples with gloves and take care to avoid cross contamination between samples and especially between cases. Samples must be processed and examined in a timely manner (within 24 hours of collection) so sample integrity is preserved.

G. INTERPRETATION

Presumptive diagnosis is based on identification of multicellular myxosporidian pre-sporogonic stages (trophozoites). Visualization of prespore stages is not sufficient for definitive diagnosis. *C. shasta* spores are 14-23 µm long and 6-8 µm wide at the suture line. The end each spore is rounded and reflected posteriorly and the suture line is distinct.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 308, Field Collection Of Ceratonova shasta Samples
4. SOP# 302, General Necropsy and Sample Collection for Inspections
5. SOP# 821, Phosphate Buffered Saline, PBS
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

Virology 600

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Cell Culture and Seeding Plates**	
SOP #: 601	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the method of splitting and maintaining various fish cell lines used in virology testing. A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. HBSS: Hank's Balanced Salt Solution
2. FBS: Fetal bovine serum
3. DMEM: Dulbecco's Minimum Essential Media
4. DMEM5: Dulbecco's Minimum Essential Media with 5% FBS and glutamine
5. DMEM10: Dulbecco's Minimum Essential Media with 10% FBS and glutamine
6. TM: transport media
7. CHSE-214: Chinook Salmon Embryo cell line
8. EPC: Epithelioma papillosum cyprini cell line
9. BF-2: Bluegill Fry cell line
10. FHM: Fathead Minnow cell line
11. CCO: Channel Catfish Ovary cell line

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Centrifuge and necessary rotors
2. Clean hood
3. 12 mm tubes with 0.8 mL TM
4. Vortex
5. Incubators
6. Refrigerators
7. 24-well plates
8. Cell culture flasks
   - Small flasks (T25)
   - Medium flasks (T75)
   - Large flasks (T150)
9. Autoclave waste bags
10. Stock and working media
    - 10X DMEM
    - DMEM10
    - DMEM5
    - 10X HBSS
    - 1X HBSS

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- TM
11. Trypsin
12. Cell scraper
13. Pipettes and tips
14. Serological pipettes and pipette aid
15. Ziplock bags
16. 70% isopropyl alcohol
17. Bleach
18. Paper towels

E. PROCEDURES

1. General considerations:
   - Prepare stock and working media as described in associated media SOPs: 10X DMEM, DMEM10, and DMEM5[1]; 10X HBSS and 1X HBSS[2]; 0.05% Trypsin solution[3]; TM[4].
     - Media with glutamine (i.e., DMEM5 and DMEM10) is only good for ~30 days, change out or add glutamine accordingly.
     - Thaw 0.05% trypsin solution.
   - Split cell lines every 10-14 days to maintain viability and promote health growth.
   - Store old cells at 4°C for ~6 months
   - Split cells and seed plates from cell layers that are 80-100% confluent.
2. Splitting cell lines:
   - Work in a clean hood.
     - Turn on UV light for at least 10 minutes prior to working in hood.
     - Wipe down all surfaces of hood and bench top with 70% isopropyl alcohol prior to working in hood.
   - Place cells and DMEM10 into 25°C incubator to warm for 10-30 minutes.
     - Decant spent growth media from cell cultures one cell line at a time.
   - Gently add 0.05% trypsin solution to the side of the flask so as not to disrupt cell layer.
     - Small flasks (T25): Wash twice with 1 mL 0.05% trypsin solution and add 0.6 mL 0.05% trypsin solution for trypsinization.
     - Medium flasks (T75): Wash twice with 2 mL 0.05% trypsin solution and add 2 mL 0.05% trypsin solution for trypsinization.
     - Large flasks (T150): Wash twice with 3 mL 0.05% trypsin solution and add 3 mL 0.05% trypsin solution for trypsinization.
   - Let cells sit in trypsin solution for 1-5 minutes.
     - Check cells after 1 minute. Gently tap the side of the flask and look under microscope for the cell layer to begin lifting with no major clumping. If adherence persists, wait about 1-3 more minutes and check again.
     - After 10 minutes, if a good portion of cells are still adhering to the flask, use a cell scraper to gently lift off the remaining cells.
   - Add 5-6 mL DMEM10 media to flask and triturate 20-30 times to dissociate clumping cells.
     - Calculate and know split ratios, and split cells according to table below.

ORIGINAL

    - Add fresh DMEM10 in accordance to the split ratios described below. For example, if you initially added 5 mL of DMEM10 and you have a split ratio of 1:6, add 25 mL of additional fresh DMEM10 to your flask.
    - Move quickly so the cells do not adhere to the flask and disrupt split ratio.
    - Split ratios are calculated from cm^2 not media volume: thus splitting the entire contents of a T25 into a T75 is a 1:3 split no matter how much volume of media was used.

Common Name	Split Ratio	Flask Type	Optimal Temp. (°C)	Morphology
EPC	1:6-8	BD Falcon	20-25	Epithelial
CHSE-214	1:4-6	BD Falcon	15-20	Epithelial
BF-2	1:6-10	Corning Costar	25-30	Fibroblast
FHM	1:4-6	BD Falcon	25-30	Epithelial
CCO	1:10-20	Corning Costar	25-30	Fibroblast

  - Add newly prepared media containing cells (from previous step) to new cell culture flasks.
    - Small flask (T25): add 7-10 mL.
    - Medium flask (T75): add 18-25 mL.
    - Large flask (T150): add 35-50 mL.
    - Be sure to add media to the appropriate side of the flask to ensure cell adherence
  - Set flasks on level surface in 22-25°C incubator for 30-60 minutes to allow cells to adhere.
    - Transfer old flasks (stock cells) to 4°C refrigerator.
    - Check fresh flasks under microscope for adherence of cells and move flasks to appropriate temperature incubators.
      - Warm water cell lines: 22-25°C.
      - Cold water cell lines: 15°C.
  - Repeat steps every 7-14 days to maintain cell propagation.
  - If not used, dispose of stock cells at 4°C after 6 months.
3. Seeding plates:
  - If more than one flask of the same cell line is needed, triturate each flask separately and then combine into a stock cell culture.
    - Each 24-well plate will require 12 mL of cell suspension (0.5 mL suspended cells/well).
    - Prepare appropriate volume of cell suspension needed for quantity of plates/wells required. Accommodate for pipetting error.
  - Seed plates with 0.5 mL of cell suspension per well. Any extra cell suspension may be added to a T25 if desired.
  - Place seeded plates on top shelf in 22-25°C incubator overnight.
    - Top shelf is reserved for tissue cell culture and bottom shelves are reserved for plates inoculated for virology.
  - Check for confluence in every well of seeded plates before inoculation[5].

F. QUALITY CONTROL

ORIGINAL

- Cell culture is sensitive to contamination. Aseptic techniques should be utilized at all times.
- All working areas should be cleaned with 70% isopropyl alcohol before and after performing this protocol.
- The laminar flow hood should also be disinfected using UV sterilization 15-30 minutes before and after performing this procedure.
- Do not use cell culture flasks if flasks are damaged and/or cell monolayer is disrupted.
- If cells appear abnormal, retrieve new stock from cryogenic storage and repropagate[6].
- If cells continue to appear abnormal, perform mycoplasma testing[7].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 809, Dulbecco's Minimal Essential Media (DMEM)
2. SOP# 813, Hank's Balanced Salt Solution (HBSS)
3. SOP# 835, Trypsin Solutions
4. SOP# 834, Transfer Media (TM)
5. SOP# 603, Plate Inoculation
6. SOP# 605, Cell Culture Cryogenic Storage
7. SOP# 607, Mycoplasma Testing
8. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
9. Terrance Ott (2001). Tissue Culture of Fish Cell Lines (Chapter 10). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet http://www.r1.fws.gov/canvfhc/nwfhsman.htm.
10. Ken Wolf (1988). The viruses and viral diseases of fish. Cornell University Press: Ithica, NY.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Tissue Processing for Virology**	
SOP #: 602	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the method to process various tissue samples for virology testing.

B. DEFINITIONS

1. HBSS: Hank's Balanced Salt Solution
2. FBS: Fetal bovine serum
3. DMEM: Dulbecco's Minimum Essential Media
4. DMEM5: Dulbecco's Minimum Essential Media with 5% FBS and glutamine
5. DMEM10: Dulbecco's Minimum Essential Media with 10% FBS and glutamine
6. TM: transport media
7. CHSE-214: Chinook Salmon Embryo cell line
8. EPC: Epithelioma papillosum cyprini cell line
9. BF-2: Bluegill Fry cell line
10. FHM: Fathead Minnow cell line
11. CCO: Channel Catfish Ovary cell line
12. K/S: Kidney/spleen samples
13. WF: Whole fish samples
14. WV: Whole viscera samples
15. OF: Ovarian fluid samples

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Tissue samples in TM
2. 5 mL tubes empty and prepared with TM
3. Clean hood
4. Stomacher
5. Stomacher bags
6. Ice packs
7. Tupperware
8. Kimwipes
9. Scale
10. 1X HBSS
11. Gentamycin
12. Sterile beaker
13. 5 mL tubes
14. Tube rack
15. Centrifuge and necessary rotors

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16. Pipettors and tips
17. Vortex
18. Autoclave bags
19. Serological pipettes and pipette-aid
20. 70% Isopropyl alcohol
21. Bleach

E. PROCEDURES

1. Processing tissues:
   - Load a 5 mL tube rack with an equal number of empty 5 mL tubes and 5 mL dilution tubes containing appropriate volume of TM[1].
     - K/S: 2 mL TM
     - WF: 2 mL TM
     - WV: 180 µL TM
     - Label both empty 5 mL tubes and 5 mL dilution tubes with lot number and sample number (i.e., 1-12).
   - Within 72 hours of collection[2] (preferably within 48 hours), remove samples from 4°C, add entire contents of collection tube to a stomacher bag.
     - Decant media from stomacher bag into autoclave waste.
     - Place tissue remaining in stomacher bag on ice.
     - Repeat for all samples.
   - Weigh each tissue sample (in stomacher bag) and add appropriate volume of 1X HBSS[3].
     - 1X HBSS to be added 1:10 m/v.
   - Stomach tissue samples (up to 3 separate bags at a time) in stomacher for 30 seconds.
     - Transfer tissue homogenate to a pre-labeled empty 5 mL tube.
     - Only work on one lot at a time.
   - Centrifuge on program 1 (3000 x g, 15 minutes, 4°C).
   - Dilute samples by adding the appropriate amount from the 1:10 dilution into the corresponding dilution tube.
     - K/S: 1 mL of 1:10 diluted sample/2mL TM = 1:30 final dilution.
     - WF: 0.5 mL of 1:10 diluted sample/2mL TM = 1:50 dilution.
     - WV: 0.2 mL of 1:10 diluted sample/ 180 µL TM = 1:100 final dilution.
     - Samples collected from June suckers: 1:200 final dilution required to avoid excessive cell toxicity problems.
     - These dilutions have been worked out in this lab for the cell lines being used by trial and error; however, a new lab technician as well as the current should always validate existing lab techniques on an ongoing basis.
   - Vortex each diluted sample.
     - Store original undiluted sample on bottom shelf of sample refrigerator until assay completion.
   - Incubate diluted samples at 15°C for ~2 hours or at 4°C for 12-24 hours.
   - After incubation, centrifuge diluted samples on program 1 (3000 x g, 15 minutes, 4°C).
2. Processing ovarian fluid samples:
   - Load a 5 mL tube rack with an equal number of empty 5 mL tubes and 5 mL dilution tubes containing 2 mL TM.
   - Within 72 hours of collection (preferably 48 hours), remove OF samples from 4°C and centrifuge on program 1 (3000 x g, 15 minutes, 4°C).

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- If samples are not processed within 6 hours of collection add 1 mL of gentamycin to reduce bacterial growth.
- Dilute 1:5 v/v (OF:TM).
  - Add 0.5 mL OF to 2 mL TM.
  - Vortex.
  - Store original undiluted sample on bottom shelf of sample refrigerator.
- Work with one lot at a time if collection has more than one lot and one collection site at a time if more than one site is inspected in the same time frame.
- Incubate diluted samples at 15°C for ~2 hours or at 4°C for 12-24 hours.
- After incubation, centrifuge diluted samples on program 1 (3000 x g, 15 minutes, 4°C).

F. QUALITY CONTROL

- Work with one lot at a time to prevent cross contamination between groups.
- Aseptic techniques should be utilized at all times.
- All working areas should be cleaned with 70% isopropyl alcohol before and after performing this protocol.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 834, Transfer Media (TM)
2. SOP# 306, Collection of Field Samples for Virology
3. SOP# 813, Hank's Balanced Salt Solution (HBSS)
4. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
5. Ray Brunson (2001). Virology (Chapter 11). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet: http://www.r1.fws.gov/canvfhc/nwfhsman.htm.
6. Scott LaPatra (2003). General Procedures for Virology (Chapter 2.1). In Suggested Procedure for the Detection and Identification of Certain Finfish and Shellfish Pathogens. Blue Book 5th Edition. Fish Health Section, American Fisheries Society. CD-ROM.
7. Theodore Meyers, William Batts and James Winton (2001). Corroborative Testing of Viral Isolates (Chapter 12). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet: http://www.r1.fws.gov/canvfhc/nwfhsman.htm.
8. Terrance Ott (2001). Tissue Culture of Fish Cell Lines (Chapter 10). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet ttp://www.r1.fws.gov/canvfhc/nwfhsman.htm.
9. Ken Wolf (1988). The viruses and viral diseases of fish. Cornell University Press: Ithica, NY.
10. P. T. K. Woo & D. W. Bruno, editors. (1999). Fish Diseases and Disorders: Viral, Bacterial, and Fungal Infections. Vol. 3 (Chapters 1-5). CABI Publishing: New York.

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Plate Inoculation**	
SOP #: 603	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods of inoculating seeded 24-well plates for standard cell culture.

B. DEFINITIONS

1. HBSS: Hank's Balanced Salt Solution
2. FBS: Fetal bovine serum
3. DMEM: Dulbecco's Minimum Essential Media
4. DMEM5: Dulbecco's Minimum Essential Media with 5% FBS and glutamine
5. DMEM10: Dulbecco's Minimum Essential Media with 10% FBS and glutamine
6. TM: transport media
7. CHSE-214: Chinook Salmon Embryo cell line
8. EPC: Epithelioma papillosum cyprini cell line
9. BF-2: Bluegill Fry cell line
10. FHM: Fathead Minnow cell line
11. CCO: Channel Catfish Ovary cell line
12. K/S: Kidney/spleen samples
13. WF: Whole fish samples
14. WV: Whole viscera samples
15. OF: Ovarian fluid samples
16. CPE: Cytopathic effect
17. BP: Blind pass
18. PS: Penicillin/streptomycin solution

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Kimwipes
2. 1X HBSS
3. DMEM5
4. TM
5. PS solution
6. Sterile beaker
7. Centrifuge and necessary rotors
8. Pipettors and tips
9. Vortex
10. Plate shaker
11. Seeded 24-well plates
12. Autoclave waste bags

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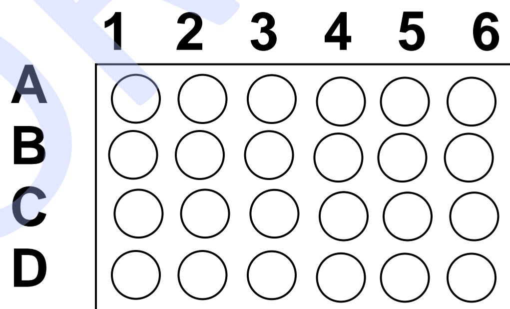
13. Serological pipettes and pipette-aid
14. 70% isopropyl alcohol

E. PROCEDURES

1. Inoculation of 24-well plates (primary inoculation):
   - Work in a clean hood.
     - Turn on UV light for at least 10 minutes prior to working in hood.
     - Wipe down all surfaces of hood and bench top with 70% isopropyl alcohol prior to working in hood.
   - Warm DMEM5[1] media to 15°C.
     - Can either put DMEM5 at 15°C overnight or at 22°C for about an hour.
   - Remove required number of pre-seeded plates[2] (80-100% confluent) and processed samples[3] from respective incubators and place in clean hood.
     - One 24-well plate will hold 12 samples (or one full lot from an inspection).
     - Each sample is inoculated in duplicate onto at least two cell lines.
       - Diagnostic samples are inoculated onto all cell lines and preferably incubated at two dilutions and two temperatures. Check with Fish Health Director for further instructions on diagnostic samples.
       - Standard cell lines and incubation temperatures:
         1) Salmonids (Cold water): CHSE, EPC, and FHM at 15°C.
         2) Non-Salmonids (Warm water): CHSE and FHM at 15°C; EPC, FHM, and BF2 at 25°C.
   - Decant media from each plate per cell line and label plate with lot number, initials and date of inoculation.
   - Add 100 µL of each processed sample in duplicate to each cell line.
     - 24-well plates are stamped during manufacture with rows A-D and columns 1-6. Wells A1 and A2 should be duplicates, wells B1 and B2 should be duplicates and so on.



     - Repeat previous steps for subsequent lots obtained from the same collection site. If inoculating from another collection site, decontaminate and run UV light in hood between sites.
     - Place processed sample tubes at 4°C until assay completion.
   - Inoculate a negative control (in duplicate) in every plate set and use a negative control on every plate whenever possible.

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        - A plate set is a series of 24-well plates inoculated at the same time originating from one flask or a combination of flasks (i.e. two T25s of CHSE are combined to seed four 24-well plates, or one T75 is used to seed five 24-well plates).
        - Inoculate negative controls (use 1X HBSS[4] into TM[5]) with the same dilution as samples.
    - Once inoculated, incubate plate(s) on plate shaker for 30-60 minutes at 15°C.
        - This step will not affect adsorption to cell line, thus incubating at this temperature is sufficient for cell lines that will require a warmer temperature for incubation in following steps.
    - After incubation, place plate(s) in clean hood and add 0.5 mL DMEM5 to each well.
        - Do not touch tip of pipette to edge of wells.
        - Change pipettes between every plate.
    - Place plates in a Ziploc bag according to collection number. .
    - Place plates at appropriate incubation temperatures.
        - Incubate all salmonid (cold water) samples at 15°C.
        - Non-salmonid (warm water) sample incubation temperatures are dictated by the holding temperature fish are kept at or temperature of receiving water (ideally if holding and receiving water temperatures are different two incubation temperature are recommended, thus twice the plates inoculated).
    - Using an inverted microscope, check plates within 24 hours of inoculation for toxicity and every other day thereafter for CPE[6] (minimum of 2 days per week).
        - To best screen plates use low power (40X) and if CPE is observed switch to higher power to discern changes to cell morphology.
        - Mark suspect wells for reinoculation.
    - Perform a blind pass at 10-14 day post inoculation or soon if any changes in cell morphology or metabolism occur.
2. Blind passing (to occur 10-14 days post primary inoculation):
    - This step is to dilute out possible viral expression inhibitors and to allow possible early viral replication.
    - Work in clean hood.
        - Turn on UV light for at least 10 minutes prior to working in hood.
        - Wipe down all surfaces of hood and bench top with 70% isopropyl alcohol prior to working in hood.
    - Assemble 5 mL tubes and label with BP, cell line taken from and tube number.
        - Tube numbers correspond to pool numbers. BP tube 1 = up to 5 wells.
        - When reinoculating because CPE is observed, label 5 mL tubes with R (for reinoculation), cell line take from and well/sample number. R tube 1 = one well displaying CPE.
    - Transfer contents from 24-well plate to 5 mL collection tubes.
        - Use a sterile transfer pipette and scrape cell layer and transfer well contents to 5 mL tube.
        - Blind passes: up to 5 wells can be combined (primary = 5 fish pooled, BP = up to 25 fish pooled).
        - For reinoculations, one sample per tube.
        - In this lab, 4 wells are combined for a complete inspection (12 pools) and adjusted accordingly for incomplete or partial inspections. Diagnostic cases are combined for BP on a case-by-case basis, but should generally be passed individually if samples are from wild stocks and combined for hatchery stocks.

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- Centrifuge on program 1 (3000 x g, 15 minutes, 4°C).
- Inoculate 100 µL of supernatant to fresh seeded 24-well plate, in duplicate as described above for primary inoculation.
  - If bacterial or fungal contamination is suspected, run supernatant through 0.45µ syringe filter prior to inoculation, and/or add 2X pre-prepared PS media.
- Add 0.5 mL DMEM5 to each well.
  - Do not touch tip of pipette to edge of wells.
  - Change pipettes between every plate.
- Keep primary plates for a minimum of 21 days post inoculation and observe every other day before discarding.
- Observe primary plates from diagnostic cases for 28 days.
- Observe blind pass/reinoculation plates every other day for 7 days.

F. QUALITY CONTROL

- Autoclave all biohazard bags after primary, blind pass or re-inoculation are complete.
- Wipe all surfaces with 70% isopropyl alcohol before and after each use.
- When working on a diagnostic case, pre- and post-clean all surfaces with a fresh bleach solution. However, use sparingly in virology lab as excess fumes and residue can negatively impact cell growth.
- UV sterilize hood and clean room for a minimum of 30 minutes before and after inoculation.

G. INTERPRETATION

Be sure to examine cell cultures appropriately[6].

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 809, Dulbecco's Minimal Essential Media (DMEM)
2. SOP# 601, Cell Culture and Seeding Plates
3. SOP# 602, Tissue Processing for Virology
4. SOP# 813, Hank's Balanced Salt Solution (HBSS)
5. SOP# 834, Transfer Media (TM)
6. SOP# 604, Cell Examination and Cytopathic Effect
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
8. Ray Brunson (2001). Virology (Chapter 11). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet: http://www.r1.fws.gov/canvfhc/nwfhsman.htm.
9. Scott LaPatra (2003). General Procedures for Virology (Chapter 2.1). In Suggested Procedure for the Detection and Identification of Certain Finfish and Shellfish Pathogens. Blue Book 5th Edition. Fish Health Section, American Fisheries Society. CD-ROM.
10. Theodore Meyers, William Batts and James Winton (2001). Corroborative Testing of Viral Isolates (Chapter 12). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet: http://www.r1.fws.gov/canvfhc/nwfhsman.htm.

ORIGINAL

11. Terrance Ott (2001). Tissue Culture of Fish Cell Lines (Chapter 10). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet ttp://www.r1.fws.gov/canvfhc/nwfhsman.htm.
12. Ken Wolf (1988). The viruses and viral diseases of fish. Cornell University Press: Ithica, NY.
13. P. T. K. Woo & D. W. Bruno, editors. (1999). Fish Diseases and Disorders: Viral, Bacterial, and Fungal Infections. Vol. 3 (Chapters 1-5). CABI Publishing: New York.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Cell Examination and Cytopathic Effect**	
SOP #: 604	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the method to examine cellular changes and identify cytopathic effect (CPE) associated with fish viruses.

B. DEFINITIONS

1. CPE: Cytopathic effect
2. CHSE-214: Chinook Salmon Embryo cell line
3. EPC: Epithelioma papillosum cyprini cell line
4. BF-2: Bluegill Fry cell line
5. FHM: Fathead Minnow cell line
6. CCO: Channel Catfish Ovary cell line
7. OF: Ovarian fluid

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Inverted microscope
2. Inoculated cell culture plates
3. Kimwipes

E. PROCEDURES

1. Cell examination:
   - Inoculated plates need to be examined the day following inoculation to preclude toxicity effects that may mimic CPE.
     - This is especially important with plates inoculated from OF's or whole fish homogenates.
     - Thereafter, examine the plates every other day for CPE.
   - Place plate on inverted microscope and examine each well of the 24-well plate using the 4x objective and the 10x ocular (40x magnification).
     - Adjust the light source intensity and the microscope diaphragm to give desired contrast.
2. Cytopathic effect (CPE):
   - CPE - Changes in the morphology and metabolism of tissue culture cells due to suspected viral infection or toxic agent.
     - See virus-specific CPE characteristics in associated documents below, specifically the American Fisheries Society-Fish Health Section Blue Book[1].
   - Changes to look for in inoculated cells:
     - Plaques:
       1) Focal area of CPE assumed to be initiated by an infectious virion.

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- Toxicity:
  1) Changes in the morphology and metabolism of tissue culture cells due to toxic substances (bacterial or fungal contamination, improper glassware cleaning, improper media preparation, etc.) in medium or inoculum.
  2) Toxicity may reduce or prevent viral replication.
  3) In most cases, toxicity occurs within one day and CPE is usually slow developing.
  4) Bacterial and fungal contaminants are implicated when media is turbid or visible contaminant colonies are apparent.
  5) There are exceptions to all rules, so be aware that inoculum with high titers of virus can affect the cell layer within 24 hours (i.e. IPNV at 22°C) and sometimes toxicity can mimic viral CPE.
  6) Always check with Fish Health Director for clarification.
- Drying of the cell layer:
  1) After inoculating plates, if the plates are not rotated (on plate shaker) at least every 10 minutes the middle of the well will dry creating a clear space (with some cell debris) surrounded by healthy cells. This can be observed with 6-10 hours.
- Overgrowth of cell layer:
  1) Cell lines are immortal cells and do not produce the chemicals necessary to prevent cells from growing on top of each other.
  2) Overgrowth is usually observed when cells are healthy and not split after 7-10 days at 15°C and 3-7 at 22°C.
- Cell layer contraction from the well sides:
  1) Commonly seen in inoculated BF2s older than 7-10 days incubated at 22°C.
- Focal areas with excess cells:
  1) Usually caused from not breaking up cells properly when seeding 24-well plates.
  2) Older cell layers of BF2s can resemble this after 7-10 days at 15°C and 3-7 at 22°C.
  3) Observe under higher power to rule-out syncytia.
- Cell layer with a "lattice" appearance:
  1) This can occur with CHSE-214 and can be observed prior to inoculation.
  2) Do not inoculate, reseed plates.

3. Confirmation of viral infection:
   - When a well from the primary inoculation, re-inoculation or blind pass is suspected of viral CPE it is re-inoculated onto fresh cells in at least two 25 cm^2 flasks (if CPE is in CHSE-214, CHSE-214 are used for passage, etc.). 25 cm^2 flasks are used because the higher volume of media overlaid after inoculation is needed for confirmatory assay (usually PCR), EM and cryopreservation.
   - A minimum of two flasks are used so that the contents of one can be frozen back for reference.
   - After CPE develops, scrape flask and send a 1.0 mL aliquot to be examined using the appropriate confirmation method.
     - Consult Fish Health Director for further instructions.

F. QUALITY CONTROL

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- Autoclave all biohazard bags after primary, blind pass or re-inoculation are complete.
- Wipe all surfaces with 70% isopropyl alcohol before and after each use.
- When working on a diagnostic case, pre- and post-clean all surfaces with a fresh bleach solution. However, use sparingly in virology lab as excess fumes and residue can negatively impact cell growth.
- UV sterilize hood and clean room for a minimum of 30 minutes before and after inoculation.

G. INTERPRETATION

See virus-specific CPE characteristics in associated documents below, specifically the American Fisheries Society-Fish Health Section Blue Book.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
2. Ray Brunson (2001). Virology (Chapter 11). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet: http://www.r1.fws.gov/canvfhc/nwfhsman.htm.
3. Scott LaPatra (2003). General Procedures for Virology (Chapter 2.1). In Suggested Procedure for the Detection and Identification of Certain Finfish and Shellfish Pathogens. Blue Book 5th Edition. Fish Health Section, American Fisheries Society. CD-ROM.
4. Theodore Meyers, William Batts and James Winton (2001). Corroborative Testing of Viral Isolates (Chapter 12). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet: http://www.r1.fws.gov/canvfhc/nwfhsman.htm.
5. Terrance Ott (2001). Tissue Culture of Fish Cell Lines (Chapter 10). In National Wild Fish Health Survey: Laboratory Procedure Manual edited by Kimberly True. Fish Hatchery Section, United State Fish and Wildlife Service. Published on the Internet ttp://www.r1.fws.gov/canvfhc/nwfhsman.htm.
6. Ken Wolf (1988). The viruses and viral diseases of fish. Cornell University Press: Ithica, NY.
7. P. T. K. Woo & D. W. Bruno, editors. (1999). Fish Diseases and Disorders: Viral, Bacterial, and Fungal Infections. Vol. 3 (Chapters 1-5). CABI Publishing: New York.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Cell Culture Cryogenic Storage**	
SOP #: 605	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes a standardized method used to freeze cell lines for long-term storage.

B. DEFINITIONS

1. DMSO: Dimethyl sulfoxide
2. FBS: Fetal bovine serum
3. DMEM: Dulbecco's Minimal Essential Media
4. DMEM10: Dulbecco's Minimum Essential Media with 10% FBS and glutamine
5. TM: transport media

C. SAFETY PRECAUTIONS

- OriGen freezing media contains DMSO. Use nitrile or butadiene gloves[1] when handling media.
- Use cryo-gloves[1] when handling tubes or other items stored at -80°C.

D. EQUIPMENT AND MATERIALS REQUIRED

1. DMEM10
2. Transfer media (TM)
3. Origen DMSO Freeze Medium
4. Tissue culture cells in 75 cm^2 (T-75) flasks
5. 0.05% trypsin solution
6. Sterile cryo-vials
7. Cryo 1 freezing container
8. -80°C freezer
9. Sterile 5 mL tubes
10. Storage boxes for freezer
11. Centrifuge
12. Cryo-gloves
13. Sterile pipettes
14. Sterile transfer pipettes
15. 70% isopropyl alcohol

E. PROCEDURES

1. Use cells in log phase growth (high rate of growth), which are usually not confluent.
2. Pre-label vials with cell lines, date of freezing and initials.
3. Trypsinize[2] 75 cm^2 flask of actively growing cells.
    - Gently add 0.05% trypsin solution[3] to the side of the flask so as not to disrupt cell layer.
        - Wash twice with 2 mL trypsin solution and add 2 mL trypsin solution for trypsinization.

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    - Let cells sit in trypsin for 1-5 minutes.
4. Suspend dislodged cells in 2-3 mL of DMEM10[4] media and transfer suspension to a sterile centrifuge tube.
5. Centrifuge at 1000x g for 5 minutes.
6. Decant supernatant with sterile pipet.
7. Resuspend cell pellet in 1.5 mL of TM[5].
8. Slowly add cell suspension to 1.5 mL aliquoted OriGen freeze medium and gently triturate with sterile transfer pipette to mix.
9. Aliquot 1 mL of cell suspension into each freezing vial.
    - Cells must be allowed to equilibrate with freeze medium for 5 minutes but no longer than 10 minutes.
10. Add approximately 250 mL 70% isopropyl alcohol to Cryo 1 freezing container.
    - Place cryo vials into Cryo 1 receptacle and place at -80° C.
11. The following day, freeze half of the vials in liquid nitrogen. Leave the remainder in the -80°C freezer.
12. Test frozen cells for viability after 24 hours or within a reasonable period of time after they are frozen[6].

F. QUALITY CONTROL

- Use cells 48-72 hours post-split (log phase growth).
- Cell culture is sensitive to contamination. Aseptic techniques should be utilized at all times.
- All working areas should be cleaned with 70% isopropyl alcohol before and after performing this protocol.
- The laminar flow hood should also be disinfected using UV sterilization 15-30 minutes before and after performing this procedure.

G. INTERPRETATION

Cryopreservation of cell lines will be considered successful when a thawed vial can produce a confluent cell monolayer in a T25 flask at 48-72 hours post inoculation.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 601, Cell Culture and Seeding Plates
3. SOP# 835, Trypsin Solutions
4. SOP# 809, Dulbecco's Minimal Essential Media (DMEM)
5. SOP# 834, Transfer Media (TM)
6. SOP # 606, Thawing Frozen Tissue Culture Cells.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Thawing Frozen Tissue Culture Cells**	
SOP #: 606	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) details the recovery of cryopreserved fish cell lines to an actively growing state for use in virology testing.

B. DEFINITIONS

1. FBS: Fetal bovine serum
2. DMEM: Dulbecco's Minimum Essential Media
3. DMEM10: Dulbecco's Minimum Essential Media with 10% FBS and glutamine

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. 37°C water bath
2. DMEM10
3. Pipettes and tips
4. Small cell culture flasks (T25)
5. Cryovial with frozen cells
6. 70% isopropyl alcohol

E. PROCEDURES

1. Prepare and warm DMEM10[1] to 15°C.
2. Thawing cryopreserved cells[2]:
   - Pipet the warmed DMEM10 medium (10x the cell suspension or ≥ 10 mL) into a sterile T25 flask.
     - Volume of DMEM10 to use: 10x the cell suspension volume, or ≥ 10 mL
     - Repeat for the number of cryovials being thawed.
   - Thaw the cryovials of cells quickly in a 37°C water bath.
     - As soon as the ice has melted, remove the cryovial from the water bath and decontaminate the outside of the cryovial with 70% isopropyl alcohol.
   - Aseptically transfer the cryovial contents to the sterile T25 flask containing DMEM10 (from previous step) and gently swirl to distribute the cells.
   - Incubate for 24 hours at 25°C.
     - Examine cells for viability[3].
   - If viable, decant spent DMEM10 from T25 flask containing cell culture, and replace with fresh DMEM10.
   - Incubate an additional 24 hours at 25°C then place actively growing flasks in a 15°C incubator.
     - Continue to monitor viability and growth of cells frequently.

ORIGINAL

F. QUALITY CONTROL

- Cell culture is sensitive to contamination. Aseptic techniques should be utilized at all times.
- All working areas should be cleaned with 70% isopropyl alcohol before and after performing this protocol.
- The laminar flow hood should also be disinfected using UV sterilization 15-30 minutes before and after performing this procedure.
- Do not use cell culture flasks if flasks are damaged and/or cell monolayer is disrupted.
- If cells appear abnormal, retrieve new stock from cryogenic storage and repropagate.
- If cells continue to appear abnormal, perform mycoplasma testing[4].

G. INTERPRETATION

- Cells will be considered viable if they adhere to the bottom of the T25 flask after 24 hours at 25°C.
- Cryopreservation will be considered successful if the cells achieve an 80-100% confluent monolayer in a T25 flask after 48-72 hours at 15°C.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 809, Dulbecco's Minimal Essential Media (DMEM)
2. SOP #605, Cell Culture Cryogenic Storage
3. SOP# 605, Cell Examination and Cytopathic Effect
4. SOP# 607, Mycoplasma Testing

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Mycoplasma Testing**	
SOP #: 607	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Virology	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to detect mycoplasma contamination of cell lines using LookOut® Mycoplasma Detection kit and polymerase chain reaction (PCR).

B. DEFINITIONS

N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment [1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sigma-Aldrich LookOut® Mycoplasma Detection kit
2. JumpStart® Taq DNA Polymerase, catalog #D9307
3. 90-100% confluent cell cultures.
4. dH_2O (molecular grade)
5. Pipettes and tips
6. PCR tubes
7. Thermocycler

E. PROCEDURES

1. Preparation of sample material:
    - Transfer 100 µL of supernatant from the test culture to a PCR tube.
    - Incubate the sample at 95°C for 5 minutes.
    - Briefly centrifuge the sample supernatant to pellet cellular debris before adding to the PCR mixture.
        - The templates are stable at 2-8°C for at 1 week.
2. PCR setup:
    - Determine the total volume of Taq polymerase and rehydration buffer required for the reactions.
        - 23 µL rehydration buffer per sample (including a positive and negative control and an additional reaction volume for pipetting error).
        - 0.5 µL Taq polymerase per sample (including a positive and negative control and an additional reaction volume for pipetting error).
    - Add appropriate volume of rehydration buffer and Taq polymerase to a sterile 5 mL microcentrifugre tube.
        - Mix the tube by carefully flicking, do not vortex.

ORIGINAL

- Remove strip of Test Reaction Tubes (transparent) from bag and cut off the appropriate number of tubes for the number of samples (including controls).
- Peel off protective film and add 23 µL of the prepared Taq/ rehydration buffer solution to each Test Reaction Tube.
- Negative control/sample addition - add 2 µL of dH_2O to the negative control tube.
- Sample reaction/ sample addition – add 2 µL of sample to the sample reaction tubes.
  - Close tubes with caps provided and label appropriately.
- Positive control preparation - remove strip of positive control reaction tubes (pink) from bag and cut off the appropriate number of tubes. Peel off protective film and add 25µL of the prepared Taq/rehydration buffer solution to each tube and close with caps provided
- Mix contents of each tube thoroughly by gently flicking tubes; do not vortex.
- Incubate at room temperature for 5 minutes.

2. Running PCR:
   - No activation step is required for JumpStart Taq DNA Polymerase.
   - Thermocycler settings are as follows:
     - 1 cycle: 94°C for 2 minutes
     - 40 cycles: 94°C for 30 seconds
55°C for 30 seconds
72°C for 40 seconds
     - Hold at 4°C until ready for next step.
3. Examine PCR product by gel electrophoresis[2].

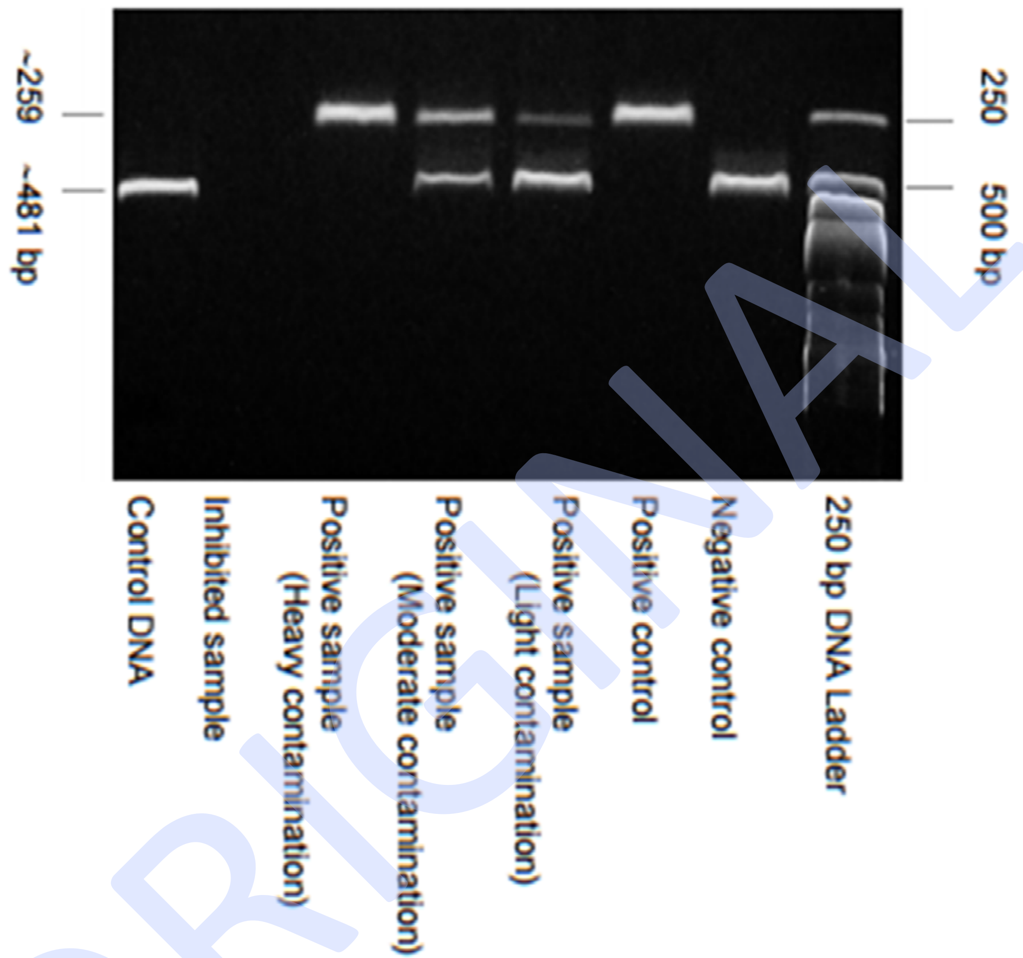
F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination.
- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

The negative control samples show a distinct 481 bp band. This internal control should appear in every lane indicating a successful PCR reaction. This band may be less intense with increased amounts of mycoplasma DNA amplicons formed. The positive control shows a band at 259 bp and sometimes an additional band at 481 bp due to the internal control. Mycoplasma positive samples show bands in the range of 260 bp ± 8 bp. See following diagram.

ORIGINAL



ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 718, Gel Electrophoresis and Visualization
3. Sigma-Aldrich LookOut® Mycoplasma PCR Detection Kit technical Bulletin MP0035

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

Molecular Techniques 700

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **DNeasy® Blood and Tissue DNA Extraction**	
SOP #: 701	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the appropriate methods of extracting DNA from blood and/or tissue using a Qiagen® DNeasy® Blood and Tissue Extraction Kit.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Wear personal protective equipment[1] when handling samples and reagents used in this protocol.
- Use caution if handling sharp dissection tools[2].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Qiagen® Dneasy® Blood and Tissue Extraction Kit (Qiagen® #69506)
2. Butcher paper
3. Bench top biohazard waste containers
4. 100% ethanol (EtOH)
5. PCR grade tips 200 µL, 1000 µL
6. 1.5 mL or 2 mL microcentrifuge tubes, PCR grade
7. Vortex
8. Centrifuge
9. Microcentrifuge tube racks
10. Sharpies
11. 10% bleach solution
12. Incubator/heat block
13. Scale
14. Dissecting tools (scissors, scalpels, forceps, etc.)
15. Cutting board
16. Tissue, blood or cell culture samples

E. PROCEDURES

1. Prepare all reagents from DNeasy® Kit.
   - Re-dissolve any precipitates in Buffer AL and Buffer ATL, if needed. Vigorously mix until solution is homogenous.
   - Add 100% EtOH to Buffer AW1 and Buffer AW2 concentrates and clearly mark date added. Pre-mixed/diluted buffers can be used if they have been clearly marked with a recent date and are not precipitated.
     - Buffer AW1: add 130 mL 100% EtOH
     - Buffer AW2: add 160 mL 100% EtOH

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2. Temper frozen tissues or cell pellets to room temperature.
3. Preheat incubator to 56°C.

4a.Tissue preparation:
- Cut tissue into small pieces and place in labeled 1.5 or 2 mL microcentrifuge tubes.
  - Spleen: ≤ 10 mg
  - Other tissues: ≤ 25 mg
- Add 180 µL Buffer ATL to each sample.
- Add 20 µL Proteinase K to each sample, and vortex.
  - Spleen: ≤ 10 mg
  - Other tissues: ≤ 25 m
- Place at 56°C until completely lysed.
  - Vortex occasionally during incubation.
  - Vortex briefly directly before proceeding to step 5.

4b.Nucleated blood preparation:
- Add 20 µL Proteinase K into a labeled 1.5 mL or 2 mL microcentrifuge tube.
- Add 5-10 µL blood to tube containing Proteinase K.
- Adjust volume to 220 µL with phosphate buffered saline (PBS).
- Proceed to step 5.

4c.Cultured cells preparation:
- Centrifuge a maximum of 5 x 10^6 cells for 5 minutes at 190 rpm.
- Re-suspend in 200 µL PBS.
- Add 20 µL Proteinase K.
- Proceed to step 5.

5. DNA extraction:
- Add 200 µL Buffer AL to each sample. Mix thoroughly by vortexing.
  - Incubate blood samples at 56°C for 10 minutes.
- Add 200 µL EtOH (96-100%) to each sample. Mix thoroughly by vortexing.
- Pipet the mixture of each sample into a DNeasy Mini spin column placed in a 2 mL collection tube.
  - Centrifuge at 8000 rpm for 1 minute.
  - Discard the flow-through and collection tube.
  - Place the spin column into a new 2 mL collection tube (provided by Qiagen).
- Add 500 µL Buffer AW1 to each sample.
  - Centrifuge at 8000 rpm for 1 minute.
  - Discard the flow-through and collection tube.
  - Place the spin column into a new 2 mL collection tube (provided by Qiagen).
- Add 500 µL Buffer AW2 to each sample.
  - Centrifuge at 14,000 rpm for 3 minutes.
  - Discard the flow-through and collection tube.
  - Place the spin column into a new labeled 1.5 or 2 mL microcentrifuge tube (NOT provided by Qiagen).
- Add 200 µL Buffer AE to the center of the spin column membrane to elute the DNA. Note: the volume of Buffer AE can be adjusted depending on concentration of DNA desired.
  - Incubate at room temperature for 1 minute.
  - Centrifuge at 8000 rpm for 1 minute.
- Optional: repeat the previous step for increased DNA yield.

ORIGINAL

F. QUALITY CONTROL

- Take caution not to cross contaminate between samples and always only work on one case/lot at a time.
- Decontaminate after processing a set of samples by wiping down all surfaces, including pipettes, with fresh 10% bleach solution followed by 70% isopropyl alcohol.

G. INTERPRETATION

DNA should be quantified[3] to ensure appropriate yields. DNA extracts are then ready for use in PCR assays; see pathogen-specific PCR SOPs for further analysis.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 205, Handling and Disposal of Sharps
3. SOP# 704, DNA Quantification Using Epoch Spectrophotometer
4. SOP# 302, General Necropsy Techniques
5. Qiagen® DNeasy® Blood and Tissue Extraction Kit manual
6. Pathogen-specific PCR SOPs

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **DNeasy® Swab Culture DNA Extraction**	
SOP #: 702	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the appropriate methods of extracting DNA from swab cultures (mucus, gill, vent, etc.) using a Qiagen® DNeasy® Blood and Tissue Extraction Kit.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Wear PPE[1] when handling samples and reagents used in this protocol.
- Use caution if handling sharp dissection tools[2].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Qiagen® Dneasy® Blood and Tissue Extraction Kit (Qiagen® #69506)
2. Butcher paper
3. Bench top biohazard waste containers
4. 100% ethanol (EtOH)
5. PCR grade tips 200 µL, 1000 µL
6. 1.5 mL or 2 mL microcentrifuge tubes, PCR grade
7. Vortex
8. Centrifuge
9. Microcentrifuge tube racks
10. Sharpies
11. 10% bleach solution
12. Incubator/heat block
13. Phosphate buffered saline (PBS)
14. Swab samples

E. PROCEDURES

1. Prepare all reagents from DNeasy® Kit.
   - Re-suspend any precipitates in Buffer AL and Buffer ATL, if needed. Vigorously mix until solution is homogenous.
   - Add 100% EtOH to Buffer AW1 and Buffer AW2 concentrates and clearly mark date added. Pre-mixed/diluted buffers can be used if they have been clearly marked with a recent date and are not precipitated.
     - Buffer AW1: add 130 mL 100% EtOH
     - Buffer AW2: add 160 mL 100% EtOH
2. Temper frozen tissues or cell pellets to room temperature.
3. Preheat incubator to 56°C.
4. DNA extraction:

- Place swab in a 2 mL microcentrifuge tube.
- Add 400 µL PBS to each sample.
- Add 20 µL Proteinase K to each sample.
- Add 400 µL Buffer AL to each sample.
  - Immediately vortex for 15 seconds. It is essential the sample and Buffer AL are mixed immediately and thoroughly.
  - Do not add Proteinase K directly to Buffer AL.
- Incubate at 56°C for 10 minutes.
- Add 400 µL EtOH (96-100%) to each sample.
  - Mix by vortexing.
- Carefully add 700 µL of the mixture from the previous step to the spin column (that is placed in a 2 mL collection tube).
  - Centrifuge at 8000 rpm for 1 minute.
  - Discard the flow-through and replace the collection tube back on the spin column.
- Repeat the previous step by adding up to 700 µL of the remaining mixture to the spin column.
  - Centrifuge at 8000 rpm for 1 minute.
  - Discard the flow-through and collection tube.
  - Place spin column in a new collection tube.
- Add 500 µL Buffer AW1 to the spin column.
  - Centrifuge at 8000 rpm for 1 minute.
  - Discard the flow-through and collection tube.
  - Place spin column in a new collection tube.
- Add 500 µL Buffer AW2 to the spin column.
  - Centrifuge at 14000 rpm for 3 minutes.
  - Discard the flow-through and collection tube.
  - Ensure that there is no carryover of AW2. It might be necessary to discard the liquid flow-through and replace the collection tube and centrifuge again.
  - Place spin column in a new labeled 1.5 mL or 2 mL microcentrifuge tube (not provided).
- Add 150 µL Buffer AE to the spin column.
  - Incubate at room temperature for 1 minute.
  - Centrifuge at 8000 rpm for 1 minute.
  - The volume of Buffer AE used may be adjusted, as elution with 100 µL of Buffer AE increases the final DNA concentration.

F. QUALITY CONTROL

- Take caution not to cross contaminate between samples and always only work on one case/lot at a time.
- Decontaminate after processing a set of samples by wiping down all surfaces, including pipettes, with fresh 10% bleach solution followed by 70% isopropyl alcohol.

G. INTERPRETATION

DNA should be quantified[3] to ensure appropriate yields. DNA extracts are then ready for use in PCR assays; see pathogen-specific PCR SOPs for further analysis.

H. ASSOCIATED DOCUMENTS/REFERENCES

ORIGINAL

1. SOP#201, Personal Protective Equipment
2. SOP#205, Handling and Disposal Of Sharps
3. SOP#704, DNA Quantification Using Epoch Spectrophotometer
4. SOP#302, General Necropsy Techniques
5. Qiagen® DNeasy® Blood and Tissue Extraction Kit manual
6. Pathogen-specific PCR SOPs

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **DNA Extraction Method from Head Tissues for *Myxobolus cerebralis* PCR**	
SOP #: 703	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the method to extract *Myxobolus cerebralis* DNA from fish tissue using the Qiagen® DNEasy® Blood and Tissue extraction kit.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when handling samples. Ensure the use of appropriate personal protective equipment[1] when handling reagents used in this protocol. Some reagents (absolute ethanol) may be harmful if ingested and/or swallowed. Use caution when handling sharp dissection tools[2].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Qiagen® DNEasy® Blood and Tissue extraction kit Qiagen# 69506
2. Autoclave bags, clear 2.0 pp VWR# 95042-563 10x15 or equivalent
3. Ethanol (EtOH), Absolute Fisher# BP2818-500 or equivalent
4. PCR grade tips 200 µL, 1000 µL
5. 2.0 mL centrifuge tubes VWR# 80077-234 or equivalent
6. Vortex
7. Centrifuge
8. Microcentrifuge tube racks
9. Butcher paper
10. Sharpies
11. 10% bleach solution

E. PROCEDURES

1. Sample Collection and Processing:
   - Reference and follow appropriate head sampling methods[3].
   - Process samples[4] using caution to avoid cross-contamination.
2. DNA Extraction (modified and optimized from Qiagen® DNEasy® Blood and Tissue Extraction Kit Manual):
   - Retrieve digested tissue samples[4].
   - Quickly vortex samples and then centrifuge for 5 minutes at full speed (14000 rpm).
   - Label a set of DNEasy® spin columns and two sets of 2 mL microcentrifuge tubes. Prepare two sets of DNEasy® collection tubes.
   - Add 300 µL of Buffer AL (included in the Qiagen® DNEasy® kit) to one set of pre-labeled microcentrifuge tubes.
   - Add 300 µL of 100% EtOH to the set of tubes now containing the AL Buffer.
   - Retrieve the digested tissue samples from the centrifuge.

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        - Add 300 µL of supernatant to the appropriately labeled tube (that contains 300 µL Buffer AL and 300 µL EtOH; store the remaining Proteinase K/ATL digest at -80°C until sample analysis is complete).
        - Immediately triturate the solution to thoroughly mix (if the solution sits for any period of time before mixing/vortexing it will precipitate).
        - Using the same tip that was used to triturate the sample, remove 450 µL (the addition of the remaining 450 µL will be added in a following step, due to the double volume we are using) of the mixture and transfer to the appropriately labeled DNEasy® spin column.
        - After adding to the spin column discard pipette tip. Make sure to use a new pipette tip for each sample.
        - Repeat until all samples have been completed.
    - Centrifuge at 8000 rpm for 1 minute.
    - Gently remove the top portion of the spin column and discard the liquid flow through into the autoclave bag.
        - Replace the collection tube back on the appropriate spin column.
        - Repeat for all samples.
    - Pipette the remaining 450 µL into the spin column and centrifuge at 8000 rpm for 1 minute.
        - Repeat for all samples.
    - Gently remove top portion of spin column and place into a new collection tube.
        - Previous collection tube containing liquid flow through can be discarded.
    - Carefully open the spin column and add 500 µL of Buffer AW1 (from Qiagen® kit).
        - Centrifuge at 8000 rpm for 1 minute.
    - Place top portion of spin column into a new collection tube.
        - Previous collection tube containing liquid flow through can be discarded.
    - Carefully open the spin column and add 500 µL Buffer AW2 (from Qiagen® kit).
        - Centrifuge at 14000 rpm for 3 minutes.
    - Place top portion of spin column in a new, pre-labeled 2 mL microcentrifuge tube and discard the collection tube containing filtrate.
    - Carefully open the spin column and add 150 µL of AE Buffer (from Qiagen® kit) to the filter and incubate at room temperature for 5 minutes.
        - Take care in pipetting to the bottom of the column without the formation of bubbles.
        - Use a new pipette tip for each sample if needed.
        - Centrifuge at 8000 rpm for 1 minute.
    - Filtered spin column can be discarded and filtrate (in the 2 mL tube) is ready to be quantified.
    - Measure DNA concentration using the Epoch plate reader[5] and store at 4°C for 30 days or less or -80°C for long term storage.
    - Decontaminate equipment and tools[6] after *M. cerebralis* processing, for complete cleaning of all surfaces and equipment used.

F. QUALITY CONTROL

- Ensure aseptic technique when processing all samples.
- Only work on one case at a time and decontaminate all equipment and lab benches before and after performing this SOP.

ORIGINAL

G. INTERPRETATION

Final concentration of DNA should be more than 100 ng/µL, extraction procedure may need to be repeated if this concentration is not reached.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP#201, Personal Protective Equipment
2. SOP#205, Handling and Disposal Of Sharps
3. SOP#310, Field Collection Of *Myxobolus cerebralis* Samples
4. SOP#505, Processing Head Samples In Preparation For DNA Extraction
5. SOP#704, DNA Quantification Using Epoch Spectrophotometer
6. SOP#506, Decontamination After *Myxobolus cerebralis* Processing
7. SOP#302, General Necropsy Techniques
8. Qiagen® DNeasy® Blood and Tissue Extraction Kit manual
9. Pathogen-specific PCR SOPs

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **RNA/DNA Quantification Using Epoch Spectrophotometer**	
SOP #: 704	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) details the process of RNA/DNA quantification from sample extracts using the Epoch spectrophotometer prior to PCR analysis.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment when handling DNA away and alcohol.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Ultrapure or molecular grade water
2. Kimwipes
3. DNA away
4. 70% Isopropyl alcohol

E. PROCEDURES

1. Turn on laptop computer in laboratory and Epoch spectrophotometer and make sure the USB cable connecting the two is plugged in.
2. Enter username "DWRUSER" and password "Pa$$w0rd" when prompted. When asked if you would like to connect to the ZENworks network, select "Cancel."
3. Double click the "Gen5 1.10" icon on the desktop and select "Take3 Session" from the New Item menu. Make sure wavelength is set to 260/280nm at 1 mg/mL.
4. The plate adaptor for the Epoch is found in the aluminum box in the laboratory on the shelf near the user manuals. Take out the adaptor and clean thoroughly with DNA away. Wipe dry with a Kimwipe.
5. Take out the small aliquot of molecular grade water contained in the adaptor case and pipet 1µl into wells A2 and A3.
6. The program requires you to blank the instrument. Push the small button to the left of the on switch on the spectrophotometer to open the tray.
7. Insert the tray and select "read blanks." If the blanks are acceptable, the illustration of the wells on the Take3 program will turn green. If the blanks exceed limitations, the wells will turn red and this process must be repeated beginning with step 4.
8. Once blanks are read, samples can now be added to the plate. Pipet 1 µl DNA or RNA extract into well B2.
9. Repeat in subsequent wells with the remainder of samples. At least 10% of total number of samples must be read. Once wells are filled, select "Read Samples."
10. An Excel spreadsheet will automatically populate when sample reading is complete. Edit this document by labeling the spreadsheet with sample numbers, sampling site, case

ORIGINAL

number, and date. Make sure this document is saved to the "G" drive with all case paperwork. As the computer is not networked, saving to a thumb drive may be necessary.

11. Once data is saved, close the Take3 Program and turn off the spectrophotometer and laptop. Clean the adaptor by wiping it with DNA away followed by 70% alcohol and place it in the adaptor case.

F. QUALITY CONTROL

- Spectrophotometer readings are considered accurate ONLY if blanks have been accepted as within limits.

G. INTERPRETATION

For PCR purposes, an optimum amount of 1.0-6.0 mg/ml of DNA or RNA should exist in each sample.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. Epoch user manual
2. Qiagen® DNeasy® Blood and Tissue Extraction Kit manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Preparation of Primers**	
SOP #: 705	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods of preparing primers for use in polymerase chain reaction (PCR) assays.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.

D. EQUIPMENT AND MATERIAL REQUIRED

1. Lyophilized or LabReady primers
2. TE buffer
3. Pipettes
4. Tips

E. PROCEDURES

1. Preparation of lyophilized primers:
   - Always take care not to contaminate original primer stock. Use filter tips and PCR-quality reagents. Work in a clean hood/bench.
   - Primers that arrive lyophilized need to be rehydrated. If needed, centrifuge to ensure that the primer pellet is at the bottom of the tube.
   - Prepare the 100 µM primer stock solution in the tube containing the pellet. This tube will be used to make working primer solutions as needed and will be stored at -20°C or below.
   - Determine how many nmoles of primer is in the tube (usually written on the tube or on information accompanying primer order).
   - Rehydrate the pellet in PCR-quality TE buffer to generate the 100 µM stock solution. An easy way to do this is to multiply the nmole value by 10 and add that volume in µL to the pellet.
     - Example: If lyophilized primer is 8.3 nmole, you will add 83 µL TE buffer to the pellet to rehydrate it. The final concentration of the primer stock is 100 µM.
   - If primers are purchased as LabReady, they will be already be at the 100 µM stock concentration and do not need to be rehydrated.
2. Diluting primers to working concentration:
   - To generate the 20 µM working dilution (which is what is generally used in primer preparation- but check pathogen specific PCR SOPs), dilute the stock solution 1:5 with TE buffer.
     - Example: 10 µL primer stock + 40 µL TE buffer = 20 µM working concentration.

ORIGINAL

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away^{TM} surface disinfectant before and after performing this protocol.

G. INTERPRETATION

See pathogen specific SOPs for further detail and laboratory analyses.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. All pathogen-specific SOPs

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Testing for DNA Contamination Associated with *Myxobolus cerebralis***	
SOP #: 706	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the quality control method to test for possible DNA contamination associated with *Myxobolus cerebralis* using polymerase chain reaction analysis (PCR).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- **DNA Away™ surface decontaminate-Potential Health Hazards:**
  - **Eyes:** - Mists or sprays of this product may moderately irritate the eyes. Symptoms of eye overexposure may include redness and tearing.
  - **Skin:** This product may irritate contaminated skin (especially in the event of prolonged overexposures). Symptoms of skin overexposure may include redness and itching.
  - **Inhalation:** Inhalation of mists or sprays from this material may cause mild to moderate respiratory irritation, coughing, burning of the respiratory tract, shortness of breath and difficulty breathing (depending on duration and concentration of exposure). Symptoms of exposure should be alleviated upon removal to fresh air. Chronic inhalation of mists or sprays may cause persistent respiratory irritation or reduced lung capacity and may cause chronic cough and breathing difficulty.
  - **Ingestion:** Ingestion is not a significant route of occupational overexposure. This material is moderately toxic by ingestion, leading to possible burns or irritation of the mouth, throat, esophagus and digestive system.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sterile cotton swab
2. 2.0 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific
3. Sterile nuclease free water – Fisher Scientific
4. DNA away™ surface decontaminate – Fisher Scientific
5. 100 µl Eppendorf™ epTIPS™ filter tips

E. PROCEDURES

1. Sample Collection:
   - The following assay should be performed on a bi-annual basis and should be scheduled for completion prior to the PCR testing of any state operated fish culture programs. Suspect contamination within the laboratory should warrant additional testing beyond the bi-annual testing schedule.
   - Wear clean gloves during the sample collection process.
   - Use a single sterile cotton applicator to thoroughly swab the laboratory surfaces listed in Table 1.

ORIGINAL

- Immediately submerse the cotton tip into a single 2 mL micro-centrifuge tube containing 1.5 mL of sterile nuclease free water.
- Break the wooden applicator into the micro-centrifuge tube so that the cotton applicator is completely submersed in the nuclease free water.
- Close the lid and collect remaining samples from other locations throughout the laboratory.
- Keep samples cool (4°C) while preparing for PCR analysis.
- If samples are not prepared for PCR analysis within 24 hours than sample collection should be repeated.
- Prepare each sample for PCR analysis by vortexing vigorously for 3-5 seconds.
- Transfer 2 µL of solution from each sample to the appropriate tubes for PCR analysis.
- PCR testing methods should include the accepted confirmation method to identify *Myxobolus cerebralis* DNA as outlined within the FHS Bluebook.

Table #1: Sample locations for QA/QC PCR analysis (*Myxobolus cerebralis*)

Sample	Sample Type	Location
1	Cotton Swab	West lab, top of (+) control hood
2	Cotton Swab	West lab, inside of (+) control hood
3	Cotton Swab	Virology lab, main counter
4	Cotton Swab	Virology lab, clean room
5	Cotton Swab	Virology lab, thermocycler
6	Cotton Swab	Virology lab, sink
7	Cotton Swab	Virology lab, hood
8	Cotton Swab	Main lab, countertop
9	Cotton Swab	Main lab, centrifuge
10	Cotton Swab	UV illumination table
11	Cotton Swab	Extraction oven
12	Cotton Swab	Gel hood
13	Cotton Swab	Gel hood counter
14	Cotton Swab	Camera

F. QUALITY CONTROL

- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat, frequently changing gloves, use of sterile micro-centrifuge tubes, pipette tips and cotton swabs.
- All working areas should also be cleaned with DNA away™ surface disinfectant before and after performing this protocol[1].

G. INTERPRETATION

- Detection of the expected 415bp amplicon is considered positive for *M. cerebralis* when visualized on an agarose gel[2]. A second 510bp band is also commonly visualized in the 2nd round of this reaction.
- Any sample not yielding this band is reported as negative.
- If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA.
- Photo document all gels and attach the photo to the case history information.
- Clean working area by applying generous amount of DNA Away and/or following disinfection procedures.

ORIGINAL

- Record results in PCR QA/QC-troubleshooting notebook and report findings to Fish Health Specialist.
- Any DNA finding should result in the area being thoroughly disinfected using a generous amount of DNA Away and the area should be retested.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 506, Decontamination After *Myxobolus cerebralis* Processing
2. SOP# 718, Gel Electrophoresis and Visualization
3. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Myxobolus cerebralis* Nested Polymerase Chain Reaction**	
SOP #: 707	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of *Myxobolus cerebralis* in extracted DNA samples by nested polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol. Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5 U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. Primers (Round 1: Tr5-16, Tr3-16; Round 2: Tr5-17, Tr3-17) – Integrated DNA Technologies
7. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
8. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
9. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
10. Thermocyler
11. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - A subsample of the extracted products should be measured[2] using a spectrophotometer to ensure that enough DNA was successfully isolated.
   - Prepare primers accordingly[3] in the laminar flow hood located within the virology clean room.

ORIGINAL

- Primary (first round primers; Andree et al. 1998)
  - Tr5-16 (Forward 1) = 5'- GCA TTG GTT TAC GCT GAT GTA GCG A-3'
  - Tr3-16 (Reverse 1) = 5'- GAA TCG CCG AAA CAA TCA TCG AGC TA-3'
- Secondary (second round primers; Andree et al. 1998)
  - Tr5-17 (Forward 2) = 5'- GCC CTA TTA ACT AGT TGG TAG TAT AGA AGC-3'
  - Tr3-17 (Reverse 2) = 5'- GGC ACA CTA CTC CAA CAC TGA ATT TG-3'
- Retrieve all necessary reagents (with the exception of Taq Polymerase. Taq must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation. All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis (primary master mix, round 1):
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
     - Primary Master Mix (50 µL reaction):
       - Sterile, molecular grade H_2O 28.6 µL
       - Buffer (5X) 10 µL
       - $MgCl_2$ (25 mM) 3 µL (final concentration 1.5 µM)
       - dNTPs (10 mM stock) 2 µL (final concentration 0.4 µM)
       - Tr5-16 Primer F (20 µM) 2 µL (final concentration 0.8 µM)
       - Tr3-16 Primer R (20 µM) 2 µL (final concentration 0. 8µM)
       - Taq Polymerase (5 units/µL) 0.4 µL (2 units per reaction)
   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
   - Place 48 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 2 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
   - Vortex and quick spin tubes.
4. Running PCR analysis (round 1):
   - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 95°C for 5 minutes (denature) 1x
     - Step 2: 95°C for 1 minute (denature)
     - Step 3: 65°C for 2.5 minute (annealing)
     - Step 4: 72°C for 1.5 minutes (extension)
     - Step 5: Return to Step 2-4 – 35x
     - Step 6: 72°C for 10 minutes (post dwell) 1x
     - Terminal: 4°C for ∞
5. Preparing secondary master mix (round 2):
   - For the nested amplification, prepare the 2nd MM, but substitute primers Tr5-17 and Tr3-17.

ORIGINAL

- This MM can be prepared at the same time as the primary MM and stored at -20°C withholding the addition of Taq until just prior to its use.
- Combine reagents into a MM solution according to recipe below.
    - Secondary Master Mix (50 µL reaction):
        - Sterile, molecular grade H_2O 28.6 µL
        - Buffer (5X) 10 µL
        - $MgCl_2$ (25 mM) 4 µL (final concentration 1.5 µM)
        - dNTPs (10 mM stock) 2 µL (final concentration 0.4 µM)
        - Tr5-17 Primer 1 (20 µM) 2 µL (final concentration 0.8 µM)
        - Tr3-17 Primer 2 (20 µM) 2 µL (final concentration 0.8 µM)
        - Taq Polymerase (5 units/µL) 0.4 µL (2 units per reaction)

6. Adding master mix and controls to PCR tubes (round 2):
    - Place 49 µL of the secondary MM into PCR strip tubes and close caps tightly to prevent evaporation. Transfer PCR tubes to sample addition hood located in the main virology laboratory.
    - Transfer 1 µL of amplified DNA from the primary reaction to the appropriately labeled strip tubes containing the secondary MM including the negative H_2O control to the appropriately labeled PCR strip tubes.
    - Vortex and quick spin tubes.
7. Running PCR analysis (round 2):
    - The parameters of the second round of this assay are identical to the first round parameters. See step 4.
8. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination.
- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization of the gel, detection of the expected 1300bp amplicon from round 1 and 415bp amplicon from round 2 is considered positive for *M. cerebralis.* Round 2 may also produce a double application consisting of a 415 and 508bp bands which is also considered positive for M. cerebralis. Any sample not yielding the appropriate band(s) is reported as negative. If there are any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotomer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 708, *Myxobolus cerebralis* Single Round PCR

ORIGINAL

6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
7. Andree, K.B., MacConnell E., and R.P. Hedrick. 1998. A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 34:145-154

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

PCR Reagents	Volume (µL)			R1 (1 rxn)	R2 (1 rxn)	(R1) x (# of samples)	(R2) x (# of samples)
			Rxn volume (µL)	50	50		
	R1 Final (per rxn)	R2 Final (per rxn)	Stock	µL	µL		
d-H_2O				28.6	28.6		
Buffer w/o Mg (X)	1	1	5	10	10		
$MgCl_2$ (mM)	1.5	2	25	3	4		
dNTP (0.4 mM/rxn)	0.4	0.4	10	2	2		
Tr3-16 (0.8 µM/rxn)	0.8	0.8	20	2	NA		
Tr5-16 (0.8 µM/rxn)	0.8	0.8	20	2	NA		
Tr3-17 (0.8 µM/rxn)	0.8	0.8	20	NA	2		
Tr5-17 (0.8 µM/rxn)	0.8	0.8	20	NA	2		
TAQ (U/µl)	0.04	0.04	5	0.4	0.4		
Total				48	49		
Template (100-300ng/rxn)				2	1		
Amount dispensed into each PCR tube prior to template addition						48	49

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Myxobolus cerebralis* Polymerase Chain Reaction; Single Round**	
SOP #: 708	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of *Myxobolus cerebralis* in extracted DNA samples by nested polymerase chain reaction analysis (PCR) as a single round assay. A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment [1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube
2. 5 mL nuclease free sterile centrifuge tube
3. Sterile nuclease free water
4. Promega GoTaq™ Flexi DNA kit
   - 500 U Flexi DNA Polymerase (5U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture
6. Primers – Integrated DNA Technologies
7. Eppendorf™ 0.2 mL PCR strip tubes
8. DNA Away™ surface decontaminate
9. Eppendorf™ epTIPS™ filter tips
10. Thermocyler
11. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation
   - It is advisable that extracted products be measured using a spectrophotometer[2] to ensure that enough DNA was successfully extracted.
   - Prepare primers[3] accordingly in the laminar flow hood located within the virology clean room.
     - Forward 1(Tr5-16) 5’- GCA TTG GTT TAC GCT GAT GTA GCG A-3’
     - Reverse 2 (Tr3-17) 5’- GGC ACA CTA CTC CAA CAC TGA ATT TG-3

ORIGINAL

- Retrieve all necessary reagents (with the exception of Taq. Taq must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation.
- All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
     - Primary Master Mix (50 µL reaction; see attached worksheet):

- Sterile, molecular grade H_2O	23.6 µL	
- Buffer (5X)	10 µL	
- $MgCl_2$ (25mM)	5 µL	(final concentration 1.5µM)
- dNTPs (10mM stock)	2 µL	(final concentration 0.2µM)
- Forward 1 (Tr5-16; 20µM)	2 µL	(final concentration 0.4µM)
- Reverse 2 (Tr3-17; 20µM)	2 µL	(final concentration 0.4µM)
- Taq Polymerase (5 units/µL)	0.4 µL	(2 units per reaction)

   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes
   - Place 45 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 5 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
   - Vortex and quick spin all tubes.
4. Running round 1 PCR analysis
   - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 95°C for 5 minutes (denature) 1x
     - Step 2: 95°C for 60 seconds (denature)
     - Step 3: 65°C for 2.5 minutes (annealing)
     - Step 4: 72°C for 1.5 minutes (extension)
     - Step 5: Return to Step 2-4 – 35x
     - Step 6: 72°C for 10 minutes (post dwell) 1x
     - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[4]

ORIGINAL

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination.
- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol[5].

G. INTERPRETATION

After performing gel electrophoresis and visualization of the gel, detection of the expected 510bp amplicon is considered positive for *M. cerebralis.* Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, DNA Quantification Using Epoch Spectrophotomer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 506, Decontamination After *Myxobolus cerebralis* Processing
6. SOP# 707, *Myxobolus cerebralis* Nested PCR
7. SOP# 703, DNA Extraction Method from Head Tissues for *M. cerebralis* PCR
8. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
9. Andree, K.B., MacConnell E., and R.P. Hedrick. 1998. A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 34:145-154

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Flavobacterium psychrophilum* Nested Polymerase Chain Reaction**	
SOP #: 709	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the method to detect the presence of *Flavobacterium psychrophilum* by nested polymerase chain reaction analysis (PCR).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol. Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile microcentrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5 U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. Primers (Round 1: Universal 1, Universal 2; Round 2: Psy1, Psy2) – Integrated DNA Technologies
7. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
8. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
9. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
10. Thermocycler
11. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured[2] using a spectrophotometer to ensure the appropriate volume of nucleic acid was isolated.
   - Prepare primers[3] in the laminar flow hood located within the virology clean room.
     - Primary (first round primers)
       - Universal 1 = 5'- AGA GTT TGA TCA TGG CTC AG-3'
       - Universal 2 = 5'- GTT TAC CTT GTT ACG ACT T-3'
     - Secondary (second round primers)
       - Psy 1 = 5'- CGA TCC TAC TTG CGT AG-3'
       - Psy 2 = 5'- GTT GGC ATC AAC ACA CT-3

- Retrieve all necessary reagents (with the exception of Taq Polymerase. Taq must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation.
- All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis (round 1):
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
     - Primary Master Mix (50 µL reaction):
       - Sterile, molecular grade H_2O 14.75 µL
       - Buffer (5X) 10 µL
       - $MgCl_2$ (25 mM) 4 µL (final concentration 2 µM)
       - dNTPs (10 mM stock) 1 µL (final concentration 0.2 µM)
       - Universal Primer 1 (20 µM) 5 µL (final concentration 2 µM)
       - Universal Primer 2 (20 µM) 5 µL (final concentration 2 µM)
       - Taq Polymerase (5 units/µL) 0.25 µL (1.25 units per reaction)
   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
   - Place 40 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 10 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
     - *Note: When performing PCR on whole cell bacteria, add these samples to the master mix in the main bacteriology laboratory or the sample hood located in the West laboratory.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
   - Vortex and quick spin all tubes.
4. Running PCR analysis (round 1):
   - The BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory is programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 95°C for 4 minutes (denature) 1x
     - Step 2: 95°C for 45 seconds (denature)
     - Step 3: 45°C for 1 minute (annealing)
     - Step 4: 72°C for 1.5 minutes (extension)
     - Step 5: Return to Step 2-5 30x
     - Step 6: 72°C for 4 minutes (final extension) 1x
     - Terminal: 4°C for ∞
5. Mixing reagents and primers for PCR analysis (round 2):
   - For the nested amplification, prepare the 2nd MM, but substitute primers Psy 1 and Psy 2.
     - This MM can be prepared at the same time as the primary MM and stored at -20°C withholding the addition of Taq until just prior to its use.
   - Combine reagents into a MM solution according to recipe below.

ORIGINAL

- Secondary Master Mix (50 µL reaction):
  - Sterile, molecular grade H_2O 23.75 µL
  - Buffer (5X) 10 µL
  - $MgCl_2$ (25 mM) 4 µL (final concentration 2 µM)
  - dNTPs (10 mM stock) 1 µL (final concentration 0.2 µM)
  - Psy 1 (20 µM) 5 µL (final concentration 2 µM)
  - Psy 2 (20 µM) 5 µL (final concentration 2 µM)
  - Taq Polymerase (5 units/µL) 0.25 µL (1.25 units per reaction)

6. Adding master mix and controls to PCR tubes (round 2):
   - Place 49 µL of the secondary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Load 1 µL of amplified DNA from the primary reaction to the appropriately labeled strip tubes containing the secondary MM including negative H_2O control to the appropriately labeled PCR strip tubes.
   - Vortex and quick spin tubes.
7. Running PCR analysis (round 2):
   - The BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory is programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 95°C for 4 minutes (denature) 1x
     - Step 2: 95°C for 45 seconds (denature)
     - Step 3: 55°C for 1 minute (annealing)
     - Step 4: 72°C for 1.5 minutes (extension)
     - Step 5: Return to Step 2-5 30x
     - Step 6: 72°C for 4 minutes (final extension) 1x
     - Terminal: 4°C for ∞
8. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization of the gel, detection of the expected 1300bp amplicon from round 1 and 1100bp amplicon from round 2 is considered positive for *F. psychrophilum.* Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or in the negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers

ORIGINAL

4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. SOP# 710, Single Round Polymerase Chain Reaction Analysis for *Flavobacterium psychrophilum*
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
8. Taylor, P.W. and J.R. Winton. 2002. Optimization of nested polymerase chain reaction assay for identification of *Aeromonas salmonicida*, *Yersinia ruckeri* and *Flavobacterium psychrophilum*. Journal of Aquatic Animal Health 14:216-224

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

		Rxn volume (µl)	50	50		
		# of samples				
PCR Reagents (final rxn concentration	**Concentration**		**R1 (1 rxn) µL**	**R2 (1 rxn) µL**	**(R1) x (# of samples)**	**(R2) x (# of samples)**
	Final Volume (per rxn)	**[Stock]**				
d-H_2O			14.75	23.75		
Buffer w/o Mg (1 X)	1	5	10	10		
$MgCl_2$ (2.0 mM)	2	25	4	4		
dNTP (200 mM/rxn)	0.2	10	1	1		
Univ 1 (100 pM/rxn)	2	20	5	NA		
Univ 2 (100 pM/rxn)	2	20	5	NA		
PSY 1 (100 pM/rxn)	2	20	NA	5		
PSY 2 (100 pM/rxn)	2	20	NA	5		
TAQ (1.25U/rxn)	0.025	5	0.25	0.25		
		Total	40	49		
		Template	10	1		
Amount dispensed into each PCR tube prior to template addition					40	49

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: *Flavobacterium psychrophilum* Polymerase Chain Reaction; Single Round**	
SOP #: 710	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the method to detect the presence of *Flavobacterium psychrophilum* by single round polymerase chain reaction analysis (PCR).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS USED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5 U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. Primers (Psy 1, Psy 2) – Integrated DNA Technologies
7. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
8. DNA away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
9. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
10. Thermocycler
11. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - A subsample of extracted DNA should be measured using a spectrophotometer[2] to ensure that enough DNA was successfully extracted.
   - Prepare primers[3] accordingly, in the laminar flood hood within the virology clean room.
     - Psy 1 = 5'- CGA TCC TAC TTG CGT AG-3'
     - Psy 2 = 5'- GTT GGC ATC AAC ACA CT-3' (Taylor and Winton 2002)
   - Retrieve all necessary reagents (with the exception of Taq Polymerase. Taq must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation. All reagents must be kept cold during preparation.
2. Mixing reagents and primers for PCR analysis:

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- Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
- Add reagents in consecutive order starting with water and ending with Taq Polymerase.
  - Master Mix (50 µL reaction):
    - Sterile, molecular grade H_2O 14.75 µL
    - Buffer (5X) 10 µL
    - $MgCl_2$ (25 mM) 4 µL (final concentration 2 mM)
    - dNTPs (10 mM stock) 1 µL (final concentration 200 µM)
    - Psy1 (20 µM) 5 µL (final concentration 2 µM)
    - Psy2 2 (20 µM) 5 µL (final concentration 2 µM)
    - Taq Polymerase (5 units/µL) 0.25 µL (1.25 units per reaction)
- Mix thoroughly.
- Return reagents to freezer immediately after use.

3. Adding master mix and controls to PCR tubes:
   - Place 40 µL of the primary MM into separate PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 10 µL of extracted sample DNA and negative H_2O control to the appropriately labeled PCR strip tubes. *Note: When performing PCR on whole cell bacteria, add these samples to the master mix in the main bacteriology laboratory or the sample hood located in the West laboratory.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
4. Running PCR analysis:
   - The BioRad MyCycler and Eppendorf™ Mastercycler located in the virology laboratory is programmed to run this reaction. Reaction conditions are the same as round 2 of the nested reaction[4] and therefore is labeled Fp2 in the BioRad MyCycler library.
   - Single Round Thermocycler conditions are as follows:
     - Step 1: 95°C for 4 minutes (denature) 1x
     - Step 2: 95°C for 45 seconds (denature)
     - Step 3: 55°C for 1 minute (annealing)
     - Step 4: 72°C for 1.5 minutes (extension)
     - Step 5: Return to Step 2-4 – 30x
     - Step 6: 72°C for 4 minutes (post dwell) 1x
     - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[5].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

ORIGINAL

After performing gel electrophoresis and visualization of the gel, detection of the expected and 1100bp amplicon from round 2 is considered positive for a single round *F. psychrophilum* PCR. Any sample not yielding the appropriate band is reported as negative. If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 709, Nested Polymerase Chain Reaction Analysis for Flavobacterium
5. SOP# 718, Gel Electrophoresis and Visualization
6. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
7. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.
8. Taylor, P.W. and J.R. Winton. 2002. Optimization of nested polymerase chain reaction assay for identification of *Aeromonas salmonicida*, *Yersinia ruckeri* and *Flavobacterium psychrophilum*. Journal of Aquatic Animal Health 14:216-224

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

PCR Reagents	Concentration: Final (per rxn)	Concentration: Stock	Single Round (1 rxn) µL	(Single Round) x (# of samples)
		Rxn volume (µL)	50	
		# of samples		
$d\text{-}H_2O$			14.75	
Buffer w/o Mg (X)	1	5	10	
$MgCl_2$ (mM)	2 mM	25	4	
dNTP (mM)	200 µM	10	1	
Forward Primer	2 µM	20 µM	5	
Reverse Primer	2 µM	20 µM	5	
Taq (U/ul)	1.2 units/rx	5 U/µL	0.25	
		Total	40	
		Template (100-300ng/rxn)	10	
			Amount dispensed into each PCR tube prior to template addition	40

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Renibacterium salmoninarum* Nested Polymerase Chain Reaction**	
SOP #: 711	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 30/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of *Renibacterium salmoninarum* in extracted DNA samples by nested polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500U Flexi DNA Polymerase (5U/µL)
   - 5X Green Flexi buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. Primers – Integrated DNA Technologies
7. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
8. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
9. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
10. Thermocyler
11. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured using a spectrophotometer[2] to ensure that enough DNA was successfully extracted.
   - Prepare primers[3] accordingly in the laminar flow hood located within the virology clean room.
     - Primary (first round primers)
       - Forward (P3) 5'- A GCT TCG CAA GGT GAA GGG -3'
       - Reverse (M21) 5'- GC AAC AGG TTT ATT TGC CGG G-3'

- Secondary (second round primers)
  - Forward (P4) 5'- AT TCT TCC ACT TCA ACA GTA CAA GG-3'
  - Reverse (M38) 5'- C ATT ATC GTT ACA CCC GAA ACC-3'
- Retrieve all necessary reagents (with the exception of Taq, which will be retrieved from the freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation.
- All reagents must be kept cold during preparation.

2. Mixing reagents and primers for round 1 PCR analysis:
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
     - Primary Master Mix (50 µL reaction; see attached worksheet):

- Sterile, molecular grade H_2O	23.6 µL	
- Buffer (5X)	10 µL	
- $MgCl_2$ (25 mM)	3 µL	(final concentration 1.5 µM)
- dNTPs (10 mM stock)	1 µL	(final concentration 0.2 µM)
- Forward 1 (P3; 20 µM)	1 µL	(final concentration 0.4 µM)
- Reverse 1 (M21; 20 µM)	1 µL	(final concentration 0.4 µM)
- Taq Polymerase (5 units/µL)	0.4 µL	(2 units per reaction)

   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
   - Place 40 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 10 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
   - Vortex and quick spin tubes.
4. Running round 1 PCR analysis:
   - Both the BioRad MyCycler and Eppendorf™ Mastercycler located in the virology laboratory are programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 94°C for 2 minutes (denature) 1x
     - Step 2: 93°C for 30 seconds (denature)
     - Step 3: 60°C for 30 seconds (annealing)
     - Step 4: 72°C for 1 minute (extension)
     - Step 5: Return to Step 2-4 – 30-40x
     - Step 6: 72°C for 10 minutes (post dwell) 1x
     - Terminal: 4°C for ∞
5. Preparing round 2 master mix:
   - For the nested amplification, prepare the 2nd MM, but substitute primers Forward 2 (P4) and Reverse 2 (M38).
     - This MM can be prepared at the same time as the primary MM and stored at -20°C withholding the addition of Taq until just prior to its use.
   - Combine reagents into a MM solution according to recipe below.

ORIGINAL

- Secondary Master Mix (50 µL reaction; see attached worksheet):
  - Sterile, molecular grade H_2O 32.6 µL
  - Buffer (5X) 10 µL
  - $MgCl_2$ (25 mM) 3 µL (final concentration 1.5 µM)
  - dNTPs (10 mM stock) 1 µL (final concentration 0.2 µM)
  - Forward 2 (P4; 20 µM) 1 µL (final concentration 0.8 µM)
  - Reverse 2 (M38; 20 µM) 1 µL (final concentration 0.8 µM)
  - Taq Polymerase (5 units/µL) 0.4 µL (2 units per reaction)

6. Adding master mix and controls to PCR tubes (round 2):
   - Place 49 µL of the secondary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Load 1 µL of amplified DNA from the primary reaction to the appropriately labeled strip tubes containing the secondary MM including negative H_2O control to the appropriately labeled PCR strip tubes.
   - Vortex and quick spin tubes.
7. Running PCR analysis (round 2):
   - The BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory is programmed to run this reaction.
   - This is the same conditions as round 1, with the exception that the second round PCR should run for only 10-20 cycles as opposed to the 30-40 cycles of the first round.
   - Thermocycler conditions are as follows:
     - Step 1: 94°C for 2 minutes (denature) 1x
     - Step 2: 93°C for 30 seconds (denature)
     - Step 3: 60°C for 30 seconds (annealing)
     - Step 4: 72°C for 1 minute (extension)
     - Step 5: Return to Step 2-4 – 10-20x
     - Step 6: 72°C for 10 minutes (post dwell) 1x
     - Terminal: 4°C for ∞
8. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination.
- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization of the gel, detection of the expected 383bp amplicon from round 1 and 320bp amplicon from round 2 is considered positive for *R. salmoninarum.* Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

ORIGINAL

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, DNA Quantification Using Epoch Spectrophotomer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNEasy® Blood and Tissue DNA Extraction

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

		Rxn volume (µL)	50	50		
		# of samples				
PCR Reagents	Concentration		R1 (1 rxn) µL	R2 (1 rxn) µL	(R1) x (# of samples)	(R2) x (# of samples)
	Final (per rxn)	Stock				
d-H_2O			23.6	32.6		
Buffer w/o Mg (X)	1	5	10	10		
$MgCl_2$ (mM)	1.5	25	3	3		
dNTP (mM)	0.2	10	1	1		
Forward 1; P3 (µM)	0.4	20	1	NA		NA
Reverse 1; M21 (µM)	0.4	20	1	NA		NA
Forward 2; P4 (µM)	0.4	20	NA	1	NA	
Reverse 2; M38 (µM)	0.4	20	NA	1	NA	
TAQ (U/µl)	0.04	5	0.4	0.4		
		Total	40	49		
		Template	10	1		
Amount dispensed into each PCR tube prior to template addition					40	49

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Viral Hemorrhagic Septicemia Virus (VHSV) Reverse Transcriptase Polymerase Chain Reaction**	
SOP #: 712	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of Viral Hemorrhagic Septicemia Virus (VHSV) in extracted RNA samples by reverse-transcriptase polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below. This assay targets the N gene of VHSV.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5 U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. AMV Reverse transcriptase (9 U/µL)
7. RNasin (39 U/µL)
8. Primers – Integrated DNA Technologies
9. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
10. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
11. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
12. Thermocyler
13. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured using a spectrophotometer[2] to ensure that enough RNA was successfully extracted. Quantification Using Epoch Spectrophotometer.

ORIGINAL

- Prepare primers[3] in the laminar flow hood located within the virology clean room.
  - Forward= 5'- GGG GAC CCC AGA CTG T-3'
  - Reverse = 5'- TCT CTG TCA CCT TGA TCC-3'
- Retrieve all necessary reagents (with the exception of Taq, which is retrieved from the freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation.
- All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis:
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
     - Master Mix (50 µL reaction, see attached worksheet):

Reagent	Volume	
- Sterile, molecular grade H_2O	21.25 µL	
- Buffer (5X)	10 µL	(final conc. 1X)
- $MgCl_2$ (25 mM)	5 µL	(final conc. 2.5 µM)
- dNTPs (10 mM stock)	1 µL	(final conc. 0.2 µM)
- AMV reverse transcriptase (9 U/µL)	0.5 µL	(final conc. 4.5 U/Rx)
- Primer Forward (20 pmoles/µL)	2.5 µL	(final conc.50 pmoles/Rx)
- Primer Reverse (20 pmoles/µL)	2.5 µL	(final conc. 50 pmoles/Rx)
- RNasin (39 U/µL)	0.25 µL	(final conc. 9.75 U/Rx)
- Taq Polymerase (5 U/µL)	0.5 µL	(2.5 units per reaction)

   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
   - Place 45 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 5 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
   - Vortex and quick spin all tubes.
4. Running PCR analysis:
   - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 50°C for 30 minutes (Reverse Transcriptase reaction) 1x
     - Step 2: 95°C for 2 minutes (denature) 1x
     - Step 3: 95°C for 30 seconds (denature)
     - Step 4: 50°C for 30 seconds (annealing)
     - Step 5: 72°C for 60 seconds (extension)
     - Step 5: Return to Step 3-5 30x
     - Step 6: 72°C for 7 minutes (final extension) 1x
     - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

ORIGINAL

- Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay. All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization, detection of the expected 811bp amplicon is considered positive for VHSV. Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

		Rxn volume (µL)	50	
		# of samples		
PCR Reagents	**Concentration**		**Single Round (1 rxn) µL**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			21.25	
Buffer w/o Mg (X)	1	5	10	
$MgCl_2$ (mM)	2.5	25	5	
dNTP (mM)	0.2	10	2.5	
AMV reverse transcriptase	4.5 U	9 U	0.5	
RNasin	9.75 U	39 U	0.25	
Forward Primer	50 pmoles	20 pmoles	2.5	
Reverse Primer	50 pmoles	20 pmoles	2.5	
Taq (U/ul)	2.5 U	5 U	0.5	
		Total	45	
		Template (100-300ng/rxn)	5	
Amount dispensed into each PCR tube prior to template addition				45

ORIGINAL

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Infectious Hematopoietic Necrosis Virus (IHNV) Reverse Transcriptase Polymerase Chain Reaction**	
SOP #: 713	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes the method to detect the presence of Infectious Hematopoietic Necrosis Virus (IHNV) in extracted RNA samples by reverse transcriptase polymerase chain reaction analysis (PCR). The primers target a portion of the G gene. A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5 U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. AMV Reverse Transcriptase
7. RNasin
8. IHNV-specific primers – Integrated DNA Technologies
9. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
10. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
11. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
12. Thermocyler
13. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured using a spectrophotometer[2] to ensure that enough RNA/DNA was successfully extracted.
   - Prepare primers[3] in the laminar flow hood located within the virology clean room.

    - Forward= 5'- AGA GAT CCC TAC ACC AGA GAC-3'
    - Reverse = 5'- GGT GGT GTT GTT TCC GTG CAA-3'
    - Emmenegger et al. 2000; Kurath et al. 2003
  - Retrieve all necessary reagents (with the exception of Taq, which must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation. All reagents must be kept cold during preparation.
2. Mixing reagents and primers for PCR analysis:
  - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
  - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
    - Master Mix (50 µL reaction, see attached worksheet):

- Sterile, molecular grade H_2O	23.75 µL	
- Buffer (10X)	5 µL	(final conc. 1X)
- $MgCl_2$ (25 mM)	5 µL	(final conc. 2.5 µM)
- dNTPs (2 mM stock)	5 µL	(final conc. 0.2 µM)
- AMV reverse transcriptase (9 U/µL)	0.5 µL	(final conc. 4.5 U/rx)
- Primer Forward (20 pmoles/µL)	2.5 µL	(final conc. 50 pmoles/rx)
- Primer Reverse (20 pmoles/µL)	2.5 µL	(final conc. 50 pmoles/rx)
- Taq Polymerase (5 U/µL)	0.5 µL	(final conc. 2.5 U/rx)
- RNasin (39 U/µL)	0.25 µL	(final conc. 9.75 U/rx)

  - Mix thoroughly using a pipette.
  - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
  - Place 45 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
  - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 5 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
  - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
  - Vortex and quick spin tubes.
4. Running PCR analysis:
  - Both the BioRad MyCycler and Eppendorf™ Mastercycler located in the virology laboratory are programmed to run this reaction.
  - Thermocycler conditions are as follows:
    - Step 1: 50°C for 30 minutes (reverse transcriptase reaction) 1x
    - Step 2: 95°C for 2 minutes (denature) 1x
    - Step 3: 95°C for 30 seconds (denature)
    - Step 4: 50°C for 30 seconds (annealing)
    - Step 5: 72°C for 1 minute (extension)
    - Step 6: Return to Step 3-5 30x
    - Step 6: 72°C for 7 minutes (final extension) 1x
    - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

ORIGINAL

- Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay. All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization of the gel, detection of the expected 693bp amplicon is considered positive for IHNV. Any sample not yielding the appropriate band(s) is reported as negative. If unusual or non-specific bands are present in the samples or in the negative controls, re-run the PCR reaction from the original template RNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

ORIGINAL

		Rxn volume (µL)	50	
		# of samples		
PCR Reagents	**Concentration**		**Single Round (1 rxn) µL**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			23.75	
Buffer w/o Mg (X)	1X	10X	5	
$MgCl_2$ (mM)	2.5 mM	25 mM	5	
dNTP (mM)	0.2 mM	2 mM	0.5	
AMV reverse transcriptase	4.5 U/rx	9 units/µL	2.5	
Forward Primer	50 pmoles/rx	20 pmoles/µL	2.5	
Reverse Primer	50 pmoles/rx	20 pmoles/µL	2.5	
Taq (U/µl)	2.5 units/rx	5 units/µL	0.5	
RNasin	9.75 units/rx	39 units/µL	0.25	
		Total	45	
		Template (100-300ng/rxn)	5	
		Amount dispensed into each PCR tube prior to template addition		49

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Infectious Pancreatic Necrosis Virus (IPNV) Reverse Transcriptase Polymerase Chain Reaction**	
SOP #: 714	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of Infectious Pancreatic Necrosis Virus (IPNV) in extracted RNA samples by reverse-transcriptase polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
5. 500U Flexi DNA Polymerase (5 U/µL)
6. 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
7. 25 mM $MgCl_2$
8. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
9. AMV Reverse transcriptase (9 U/ µL)
10. RNasin (39 U/µL)
11. IPNV-specific primers – Integrated DNA Technologies
12. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
13. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
14. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
15. Thermocyler
16. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured using a spectrophotometer[2] to ensure that enough RNA was successfully extracted.
   - Prepare primers[3] in the laminar flow hood located within the virology clean room.

- Retrieve all necessary reagents (with the exception of Taq, which must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation. All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis:
    - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
    - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
    - Mix thoroughly using a pipette.
    - Return reagents to freezer immediately after use.
        - Master Mix (50 µL reaction, see attached worksheet):
            - Sterile, molecular grade H_2O 21.25 µL
            - Buffer (5X) 10 µL (final conc. 1X)
            - $MgCl_2$ (25 mM) 5 µL (final conc. 2.5 µM)
            - dNTPs (10 mM stock) 1 µL (final conc. 0.2 µM)
            - AMV reverse transcriptase (9 U/µL) 0.5 µL (final conc. 4.5 U/Rx)
            - Primer Forward (20 pmoles/µL) 2.5 µL (final conc.50 pmoles/Rx)
            - Primer Reverse (20 pmoles/µL) 2.5 µL (final conc. 50 pmoles/Rx)
            - RNasin (39 U/µL) 0.25 µL (final conc. 9.75 U/Rx)
            - Taq Polymerase (5 units/µL) 0.5 µL (2.5 units per reaction)
            - Primers

              Forward= 5'- AAA GCC ATA GCC GCC CAT GAA C-3'

              Reverse = 5'- TCT CAT CAG CTG GCC CAG GTA C-3'
3. Adding master mix and controls to PCR tubes:
    - Place 45 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
    - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 5 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
    - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
4. Running PCR analysis:
    - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction. Thermocycler conditions are as follows:
        - Step 1: 50°C for 15 minutes (Reverse Transcriptase reaction) 1x
        - Step 2: 95°C for 2 minutes (denature) 1x
        - Step 3: 95°C for 30 seconds (denature)
        - Step 4: 50°C for 30 seconds (annealing)
        - Step 5: 72°C for 60 seconds (extension)
        - Step 5: Return to Step 3-5 35x
        - Step 6: 72°C for 7 minutes (final extension) 1x
        - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.

ORIGINAL

- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization of the gel, detection of the expected 174bp amplicon is considered positive for IPNV. Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or the negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

		Rxn volume (μL)	50	
		# of samples		
PCR Reagents	**Concentration**		**Single Round (1 rxn)**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
$d\text{-}H_2O$			21.25	
Buffer w/o Mg (X)	1	5	10	
$MgCl_2$ (mM)	2.5	25	5	
dNTP (mM)	0.2	10	2.5	
AMV reverse transcriptase	4.5 U	9 U	0.5	
RNasin	9.75 U	39 U	0.25	
Forward Primer	50 pmoles	20 pmoles	2.5	
Reverse Primer	50 pmoles	20 pmoles	2.5	
Taq (U/μl)	2.5 U	5 U	0.5	
		Total	45	
		Template (100-300ng/rxn)	5	
		Amount dispensed into each PCR tube prior to template addition		45

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Spring Viremia of Carp Virus (SVCV) Semi-Nested Reverse Transcriptase Polymerase Chain Reaction**	
SOP #: 715	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of Spring Viremia of Carp Virus (SVCV) in extracted RNA samples by a semi-nested reverse-transcriptase polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. M-MLV Reverse transcriptase (10 U/ µL)
7. RT buffer (5X)
8. RNasin (39 U/µL)
9. SVCV-specific primers (reverse transcriptase and nested primer set) – Integrated DNA Technologies
10. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
11. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
12. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
13. Thermocyler
14. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured[2] using a spectrophotometer to ensure that enough RNA was successfully extracted.

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- Prepare primers[3] in the laminar flow hood located within the virology clean room.
  - RT reaction primers
    - Forward= None
    - Reverse (SVCV R2) = 5'- AGA TGG TAT GGA CCC CAA TAC ATH CAN CAY-3'
  - First round "semi-nested" primers
    - Forward (SVCV F1) = 5'- TCT TGG AGC CAA ATA GCT CAR RTC-3'
    - Reverse (SVCV R2) = 5'- AGA TGG TAT GGA CCC CAA TAC ATH CAN CAY-3'
  - Second round "semi- nested" primers
    - Forward (SVCV F1) = 5'- TCT TGG AGC CAA ATA GCT CAR RTC-3'
    - Reverse (SVCV R4) = 5'- CTG GGG TTT CCN CCT CAA AGY TGY-3'
- Prepare reverse transcription (RT) buffer if needed.
  - 5X RT buffer: 250 mM tris pH 8.3, 375 mM KCl, 50 mM DTT, 15 mM $MgCl_2$
- Retrieve all necessary reagents (with the exception of Taq. Taq must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation.
- All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis:
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
     - Reverse Transcription Master Mix (20 µL reaction, see attached worksheet):

- Sterile, molecular grade H_2O	10 µL	
- RT buffer (5X)	4 µL	(final conc. 1X)
- dNTPs (10 mM stock)	1 µL	(final conc. 1 µM)
- M-MLV reverse transcriptase (10 U/µL)	2 µL	(final conc. 20 U/Rx)
- Primer SVCV R2 (100 pmoles/µL)	1 µL	(final conc.100 pmoles/Rx)

   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes (RT reaction):
   - Place 19 µL of the Reverse Transcription MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 1 µL of each sample (purified RNA template) including negative H_2O control to the appropriately labeled PCR strip tubes.
     - Dilute template if more than 300 ng/µL or use up to 5 µL/reaction if reading falls below 50 ng/µL.
   - The addition of positive controls should be performed in the PCR positive addition hood located in the West laboratory and should be the last step in the preparation process.
   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
   - Vortex and quick spin tubes.
4. Incubate tubes at 37°C for 1 hour.
   - This can be done in the thermocycler.
5. Preparing for first round "semi-nested" PCR:
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.

ORIGINAL

- Add reagents in order starting with dH_2O and finishing with Taq.
  - First Round Master Mix (50 µL reaction, see attached worksheet):
    - Sterile, molecular grade H_2O 36.75 µL
    - PCR buffer (10X) 5 µL (final conc. 1X)
    - $MgCl_2$ (25 mM) 5 µL (final conc. 2.5 mM)
    - dNTPs (10 mM stock) 1 µL (final conc. 0.2 µM)
    - Taq (5 U/µL) 0.25 µL (final conc.1.25 U/Rx)
- Mix thoroughly.
- Return reagents to freezer immediately after use.

6. Adding master mix and controls to PCR tubes (first round "semi-nested"):
   - Place 45.5 µL of the first round MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Add 2.5 µL of RT product for each RNA sample.
   - Add 1 µL of each of the primers SVCV F1 and SVCV R2 (primer concentration 50 pM/µL).
   - Vortex and quick spin tubes.
7. Running the first round "semi-nested" PCR:
   - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 95°C for 60 seconds (denature)
     - Step 2: 55°C for 60 seconds (annealing)
     - Step 3: 72°C for 60 seconds (extension)
     - Step 4: Return to Step 1-3 35x
     - Step 5: 72°C for 10 minutes (final extension) 1x
     - Terminal: 4°C for ∞
8. Examine PCR product by gel electrophoresis.[4]
   - If no appropriate band is seen, proceed to step 9.
     - Bands occurring at the 714 bp location in the first round are confirmatory for SVCV.
9. Preparing second round semi-nested PCR (if necessary):
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in order starting with dH_2O and finishing with Taq.
     - Second Round Master Mix (50 µL reaction, see attached worksheet):
       - Sterile, molecular grade H_2O 36.75 µL
       - PCR buffer (10X) 5 µL (final conc. 1X)
       - $MgCl_2$ (25 mM) 5 µL (final conc. 2.5 mM)
       - dNTPs (10 mM stock) 1 µL (final conc. 0.2 µM)
       - Taq (5 U/µL) 0.25 µL (final conc.1.25 U/Rx)
   - Mix thoroughly.
   - Return reagents to freezer immediately after use.
10. Adding master mix and controls to PCR tubes (second round "semi-nested"):
    - Place 45.5 µL of the second MM into PCR strip tubes and close caps tightly to prevent evaporation.
    - Add 2.5 µL of the product from the first round for each RNA sample as the template.
    - Add 1 µL of each of the primers SVCV F1 and SVCV R2 (primer concentration 50 pM/µL).

ORIGINAL

- Vortex and quick spin tubes.

11. Running the second round "semi-nested" PCR:
    - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
    - Thermocycler conditions are as follows:
        - Step 1: 95°C for 60 seconds (denature)
        - Step 2: 55°C for 60 seconds (annealing)
        - Step 3: 72°C for 60 seconds (extension)
        - Step 4: Return to Step 1-3 35x
        - Step 5: 72°C for 10 minutes (final extension) 1x
        - Terminal: 4°C for ∞
12. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

Polymerase chain reaction analysis is sensitive to contamination. Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay. All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization, detection of the expected 714 bp amplicon after the first round PCR is considered positive for SVCV. Detection of the expected 606 bp amplicon after the second round PCR is considered positive for SVCV. Any sample after the second round PCR not yielding the appropriate band(s) is reported as negative. If any unusual/non-specific bands are present in samples wells or in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheets for reaction preparation.

ORIGINAL

Reverse Transcriptase Master Mix

		Rxn volume (µL)	20	
		# of samples		
PCR Reagents	**Concentration**		**RT MM (1 rxn)**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			10	
RT buffer	1X	5X	4	
dNTP's	1 mM	10 mM	2	
M-MLV reverse transcriptase	20 U/Rx	10 U/ µL	2	
Primer SVCV R2	100 pmoles/ µL	100 pmoles/ µL	1	
		Total	19	
		Template (100-300ng/rxn)	1	
Amount dispensed into each PCR tube prior to template addition				19

Round 1 Master Mix

		Rxn volume (µL)	50	
		# of samples		
PCR Reagents	**Concentration**		**1st round MM (1 rxn)**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			36.75	
PCR buffer	1X	10X	5	
$MgCl_2$	2.5 mM	25 mM	5	
dNTP's	0.2 mM	10 mM	1	
Taq	1.25 U/Rx	5 U/ µL	0.25	
		Total	48	
		Template from RT reaction	2.5	
Amount dispensed into each PCR tube prior to template addition				45.5

ORIGINAL

Round 2 Master Mix

		Rxn volume (μL)	50	
		# of samples		
PCR Reagents	**Concentration**		**1st round MM (1 rxn)**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			36.75	
PCR buffer	1X	10X	5	
$MgCl_2$	2.5 mM	25 mM	5	
dNTP's	0.2 mM	10 mM	1	
Taq	1.25 U/Rx	5 U/ μL	0.25	
		Total	48	
		Template from round 1 reaction	2.5	
		Amount dispensed into each PCR tube prior to template addition		45.5

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Largemouth Bass Virus (LMBV) Polymerase Chain Reaction**	
SOP #: 716	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of Largemouth Bass Virus (LMBV) in extracted DNA samples by polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
   - 500 U Flexi DNA Polymerase (5U/µL)
   - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
   - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. TMAC (tetramethyl ammonium chloride)
7. LMBV-specific primers – Integrated DNA Technologies
8. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
9. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
10. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
11. Thermocyler
12. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
   - It is advisable that extracted products be measured[2] using a spectrophotometer to ensure that enough DNA was successfully extracted.
   - Prepare primers[3] in the laminar flow hood located within the virology clean room.
     - Forward = 5'- GCG GCC AAC CAG TTT AAC GCA A-3'
     - Reverse = 5'- AGG ACC CTA GCT CCT GCT TGA T-3'
     - Emmenegger et al. 2000; Kurath et al. 2003

ORIGINAL

- Retrieve all necessary reagents (with the exception of Taq, which must be retrieved from the freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation.
- All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis:
    - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
    - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
        - Master Mix (50 µL reaction, see attached worksheet):
            - Sterile, molecular grade H_2O 8.5 µL
            - Buffer (5X) 10 µL (final conc. 1X)
            - $MgCl_2$ (25 mM) 5 µL (final conc. 2.5µM)
            - dNTPs (10 mM stock) 4 µL (final conc. 0.2µM)
            - TMAC (100 µM) 20 µL (final conc. 40 µM)
            - Primer Forward (100 µM) 0.5 µL (final conc.1 µM)
            - Primer Reverse (100 µM) 0.5 µL (final conc. 1 µM)
            - Taq Polymerase (5 units/µL) 0.5 µL (2.5 units per reaction)
    - Mix thoroughly.
    - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
    - Place 49 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
    - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 1 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
    - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
    - Vortex and quick spin tubes.
4. Running PCR analysis:
    - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
    - Thermocycler conditions are as follows:
        - Step 1: 95°C for 3 minutes 15 seconds (denature) 1x
        - Step 2: 95°C for 45 seconds (denature)
        - Step 3: 60°C for 35 seconds (annealing)
        - Step 4: 72°C for 60 seconds (extension)
        - Step 5: Return to Step 3-5 35x
        - Step 6: 72°C for 7 minutes (final extension) 1x
        - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination.
- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.

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- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

G. INTERPRETATION

After performing gel electrophoresis and visualization, detection of the expected 248bp amplicon is considered positive for IHNV. Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

		Rxn volumn (µL)	50	
		# of samples		
PCR Reagents	**Concentration**		**Single Round (1 rxn) µL**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			8.5	
Buffer w/o Mg (X)	1	5	10	
$MgCl_2$ (mM)	2.5	25	5	
dNTP (mM)	0.2	10	4	
TMAC	40 µM	100 µM	20	
Forward Primer	1 µM	100 µM	0.5	
Reverse Primer	50 pmoles	20 pmoles	0.5	
Taq (U/µl)	2.5 U	5 U	0.5	
		Total	49	
		Template (100-300ng/rxn)	1	
		Amount dispensed into each PCR tube prior to template addition		49

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: ***Tetracapsuloides bryosalmonae* Polymerase Chain Reaction**	
SOP #: 717	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the procedure to detect the presence of *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKX), in extracted DNA samples by polymerase chain reaction analysis (PCR). A portion of this SOP is derived directly from the associated documents/references listed below.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use aseptic technique when preparing reaction and appropriate personal protective equipment[1] when handling reagents used in this protocol.
- Some reagents (i.e., SYBR Safe DNA gel stain) may be harmful if ingested and/or swallowed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1.5 mL nuclease free sterile micro-centrifuge tube – Fisher Scientific (Cat. # 14-380-813)
2. 5 mL nuclease free sterile centrifuge tube – Fisher Scientific (Cat. #03-391-276)
3. Sterile nuclease free water – Fisher Scientific (Cat. #BP561-1)
4. Promega GoTaq™ Flexi DNA kit – Fisher Scientific (Cat. #PR-M8295)
    - 500 U Flexi DNA Polymerase (5 U/µL)
    - 5X Green Flexi Buffer without $MgCl_2$ (contains loading buffer)
    - 25 mM $MgCl_2$
5. 10 mM DNTP mixture – Fisher Scientific (Cat. #PRU1515)
6. *T. brysalmonae*-specific primers – Integrated DNA Technologies
7. Eppendorf™ 0.2 mL PCR strip tubes – Fisher Scientific (Cat. # E0030 124 286)
8. DNA Away™ surface decontaminate – Fisher Scientific (Cat. #21-236-28)
9. Eppendorf™ epTIPS™ filter tips (Cat. # 05-403)
10. Thermocyler
11. PCR tube racks/frozen cryoracks

E. PROCEDURES

1. Reagent preparation:
    - It is advisable that extracted products be measured[2] using a spectrophotometer to ensure that enough DNA was successfully extracted.
    - Prepare primers[3] in the laminar flow hood located within the virology clean room.
        - Forward= 5'- CCT ATT CAT TGA GTA GAG A-3'
        - Reverse = 5'- GGA CCT TAC TCG TTT CCG ACC-3'
        - Kent et al. 1998

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- Retrieve all necessary reagents (with the exception of Taq, which must be retrieved from freezer immediately prior to its addition) from -20°C and place in frozen cryorack during preparation. All reagents must be kept cold during preparation.

2. Mixing reagents and primers for PCR analysis:
   - Combine all reagents into a master mix (MM) solution within a 1.5-5 mL nuclease free centrifuge tube according the recipe below.
   - Add reagents in consecutive order starting with water and ending with Taq Polymerase.
     - Master Mix (50 µL reaction, see attached worksheet):
       - Sterile, molecular grade H_2O 33.4 µL
       - Buffer (5X) 10 µL (final conc. 1X)
       - $MgCl_2$ (25 mM) 5 µL (final conc. 2.5µM)
       - dNTPs (10 mM stock) 2.5 µL (final conc. 0.2µM)
       - Primer Forward (20 pmoles/µL) 2 µL (final conc.0.8 µM)
       - Primer Reverse (20 pmoles/µL) 2 µL (final conc. 0.8 µM)
       - Taq Polymerase (5 U/µL) 0.2 µL (1 U/rx)
   - Mix thoroughly u using a pipette.
   - Return reagents to freezer immediately after use.
3. Adding master mix and controls to PCR tubes:
   - Place 48 µL of the primary MM into PCR strip tubes and close caps tightly to prevent evaporation.
   - Transfer PCR tubes to sample addition hood located in the main virology laboratory and load 2 µL of each sample including negative H_2O control to the appropriately labeled PCR strip tubes.
   - The addition of positive controls should be performed in the PCR hood located in the West laboratory and should be the last step in the preparation process.
   - Vortex and quick spin tubes.
4. Running PCR analysis:
   - Both the BioRad MyCycler and Eppendorf Mastercycler located in the virology laboratory are programmed to run this reaction.
   - Thermocycler conditions are as follows:
     - Step 1: 94°C for 3 minutes (initial denature) 1x
     - Step 2: 95°C for 1 minute (denature)
     - Step 3: 55°C for 1 minute (annealing)
     - Step 4: 72°C for 1 minute (extension)
     - Step 5: Return to Step 3-5 35x
     - Step 6: 72°C for 5 minutes (final extension) 1x
     - Terminal: 4°C for ∞
5. Examine PCR product by gel electrophoresis[4].

F. QUALITY CONTROL

- Polymerase chain reaction analysis is sensitive to contamination.
- Aseptic techniques should be utilized at all times and should include basic precautions such as the use of a clean lab coat and frequently changing gloves and pipette tips when performing this assay.
- All working areas should also be cleaned with DNA Away™ surface disinfectant before and after performing this protocol.

ORIGINAL

G. INTERPRETATION

After performing gel electrophoresis and visualization, detection of the expected 501bp amplicon is considered positive for *Tetracapsuloides bryosalmonae* . Any sample not yielding the appropriate band(s) is reported as negative. If any unusual bands are present in the samples or if bands are present in negative controls, re-run the PCR reaction from the original template DNA. Photo document all gels and attach the photo to the case history information.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 704, RNA/DNA Quantification Using Epoch Spectrophotometer
3. SOP# 705, Preparation of Primers
4. SOP# 718, Gel Electrophoresis and Visualization
5. SOP# 701, DNeasy® Blood and Tissue DNA Extraction
6. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached worksheet for reaction preparation.

		Rxn volume (µL)	50	
		# of samples		
PCR Reagents	**Concentration**		**Single Round (1 rxn) µL**	**(Single Round) x (# of samples)**
	Final (per rxn)	**Stock**		
d-H_2O			26.3	
Buffer w/o Mg (X)	1	5	10	
$MgCl_2$ (mM)	2.5	25	5	
dNTP (mM)	0.2	10	2.5	
Forward Primer	50 pmoles	20 pmoles	2	
Reverse Primer	50 pmoles	20 pmoles	2	
Taq (U/ul)	2.5 U	5 U	0.2	
		Total	45	
		Template (100-300ng/rxn)	2	
Amount dispensed into each PCR tube prior to template addition				48

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Gel Electrophoresis and Visualization**	
SOP #: 718	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Molecular Techniques	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the general methods of casting and running an electrophoresis gel. In addition, this SOP describes the procedures for proper visualization and documentation of the gel.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1] and take caution when handling gel solution after it has been microwaved, as it will be hot.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1x TBE buffer[2]
2. Agarose powder
3. Microwave
4. Sybr Safe gel stain
5. Gel casting holder and combs
6. Small flask
7. Scale
8. Weigh boat
9. Pipettors and tips
10. DNA ladder

E. PROCEDURES

1. Gel casting:

	Full sized 1.5% agarose gel	Half-sized 1.5% agarose gel
Agarose powder	1.8 g	0.9 g
1x TBE buffer	120 mL	60 mL
Sybr® Safe gel stain	6 µL	3 µL

- Set up gel holder with rubber gaskets and or/tape. Prepare gel holder with the appropriate number and size of combs needed for samples to be analyzed.
- Weigh appropriate amount of agarose powder needed for the desired size gel, add to flask.
- Add appropriate amount of 1x TBE buffer needed for the desired size gel, add to flask or beaker, swirl to mix.
- Heat in gel microwave until boiling (approx. for 1 minute). Swirl half way through to mix. Flask will be hot, use autoclave gloves when handling.

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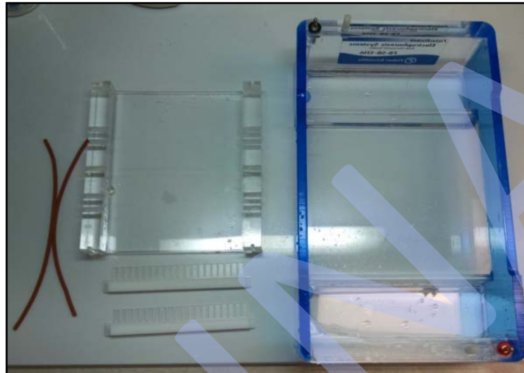
- Allow mixture to cool slightly but not solidify, add appropriate volume of Sybr® Safe gel stain and swirl flask to thoroughly mix.
- Immediately pour solution into gel casting chamber fixed with gel combs.
- Let gel set for approximately 20-30 minutes before use.
- After solution has solidified, carefully remove combs and place gel into electrophoresis chamber. Insert the gel tray into the unit so that the samples run downwards toward the red cathode in the electrophoresis unit (see photo). Ensure gel box (including both reservoirs) is filled completely with 1x TBE buffer and gel is fully submerged. *Note: red electrode in bottom right corner of unit.



2. Gel loading:
    - Load 5 µL of 1kb Plus DNA ladder into the well at both ends of each row.
    - Carefully load 6-8 µL of each sample to individual wells, ensure tip does not puncture through well and template flows and seats appropriately into each well.
    - Repeat until all samples and positive and negative controls have been loaded.
    - *Note: Promega GoTaq™ Flexi DNA kit contains tracking dye and therefore will not need to be added separately to amplified samples.
3. Electrophoresis:
    - Place the cover on the electrophoresis unit and be sure to wet electrodes with TBE buffer before securing cover. Turn on the power box (located outside of the gel loading hood), set parameters at 100V and run for 45-60 minutes. If power box displays an error code, turn off power, remove cover on gel box and re-wet electrodes and try again.
    - The tracking dye should migrate towards the red anode during electrophoresis.
    - At the end of the program an alarm will sound. Press the stop button, turn off the power and wait 5 seconds for the charge to dissipate before removing the lid to avoid electrical shock.
    - Remove the casting tray and transfer gel to the UV transilluminator.
4. UV transilluminator and gel photography/documentation:
    - Before turning on the UV source, ensure proper protective eye wear (i.e., short wave UV shielding eyewear).
    - Turn on the transilluminator and position the gel for photography. The recommended settings for the Olympus 350 are as follows:
        - Mode dial on top of camera should be set to M
        - ESP = On
        - Micro setting = Off
        - ISO = 400
        - Timer = Off
        - Flash = -0.7

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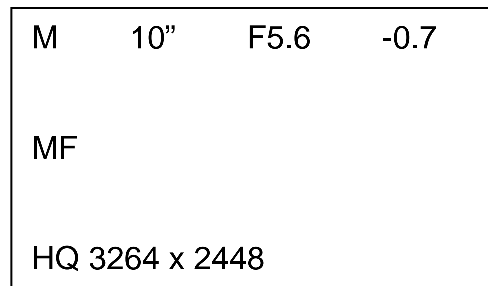
Camera view screen should resemble:



- Flash = SYNC 1
- Digital zoom = Off
- Noise reduction = On
- Focus mode = MF
- Speaker = Off
- Timelapse = Off

- Place the camera and hood over the gel, squeeze the trigger on the camera and wait ~10 seconds for picture to record.
- Transfer photograph into Photoshop and record banding pattern in relation to DNA ladder.
- Place the gel in the appropriate receptacle for incineration and clean the transilluminator using DNA Away™ surface decontaminator.
- Dispose of waste TBE from electrophoresis unit in laboratory sink. TBE buffer can be reused for approximately 1 week or 5 uses before needing to be changed.
- Bleach instruments as needed

F. QUALITY CONTROL

- Take care not to cross contaminate between samples and controls (negative and positive).

G. INTERPRETATION

See associated SOPs and pathogen specific SOPs for further analysis.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 832, 1X Tris Borate EDTA (TBE) Buffer
3. SOP# 707, *Myxobolus cerebralis* Nested PCR
4. SOP# 708, *Myxobolus cerebralis* Single Round PCR
5. SOP# 709, Flavobacterium psychrophilum Nested PCR
6. SOP# 710, Flavobacterium psychrophilum Single Round PCR
7. SOP# 711, *Renibacterium salmoninarum* Nested PCR
8. SOP# 712, Viral Hemorrhagic Septicemia Virus (VHSV) Reverse Transcriptase Polymerase Chain Reaction
9. SOP# 713, Infectious Hematopoietic Necrosis Virus (IHNV) Reverse Transcriptase Polymerase Chain Reaction
10. SOP# 714, Infectious Pancreatic Necrosis Virus (IPNV) Reverse Transcriptase Polymerase Chain Reaction
11. SOP# 715, Spring Viremia of Carp Virus (SVCV) Semi-Nested Reverse Transcriptase Polymerase Chain Reaction
12. SOP# 716, Largemouth Bass Virus (LMBV) Polymerase Chain Reaction
13. SOP# 717, Tetracapsuloides bryosalmonae Polymerase Chain Reaction
14. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

ORIGINAL

J. APENDICES

- N/A

ORIGINAL

Media 800

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Alsever's Solution**	
SOP #: 801	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to prepare Alsever's solution which is used as an anticoagulant/blood preservative.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sodium chloride (NaCl)
2. Sodium citrate ($Na_3C_6H_5O_7$)
3. Citric acid
4. Sucrose ($C_{12}H_{22}O_{11}$)
5. dH_2O
6. Glass bottle
7. Volumetric flask

E. PROCEDURES

	100 mL	250 mL	500 mL
Sodium Chloride (NaCl)	0.42 g	1.05 g	2.1 g
Sodium Citrate ($Na_3C_6H_5O_7$)	0.8 g	2.0 g	0.4 g
Citric Acid ($C_6H_8O_7$)	0.055 g	0.1375 g	0.275 g
Sucrose ($C_{12}H_{22}O_{11}$)	2.055 g	5.1375 g	10.25 g

1. Weigh reagents and add to volumetric flask, QS to appropriate volume.
2. Add stir bar and stir until dissolved.
3. Transfer to sterile glass bottle.
4. Autoclave at 121°C for 10 minutes (100 mL) or 15 minutes (500 mL).
5. Let cool, label and aliquot if needed.

F. QUALITY CONTROL

N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. Alsever, J.B., and Ainslie, R. 1941. New York State Journal of Medicine: 41(126).

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: 10% Bovine Serum Albumin**	
SOP #: 802	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods for preparing 10% bovine serum albumin for the use in pepsin-trypsin digestion (PTD) for recovery of *M. cerebralis* spores. Bovine serum albumin inactivates the enzymatic reagent trypsin and halts further digestion.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution and wear appropriate personal protective equipment[1] when handling chemicals.

D. EQUIPMENT AND MATERIALS REQUIRED

1. dH_2O
2. Bovine serum albumin
3. 1 L flask or glass bottle
4. Stir bar
5. Stir plate
6. Aluminum foil

E. PROCEDURES

Powered bovine serum albumin	52.1 g
dH_2O	QS to 500 mL

1. Place a large stir bar in a clean 1 L flask and place on stir plate, add approximately 250 mL of dH_2O to flask.
2. Weigh out 52.1 g of albumin in large weigh boat and transfer to flask on stir plate. Rinse weigh boat with dH_2O and add to flask to remove all powered residue.
3. Add remaining dH_2O to final volume of 500 mL.
4. Cover flask with foil and center on large stir plate. Spin at moderate speed until protein is completely dissolved. DO NOT HEAT.
5. Aliquot approximately 40-45 mL into sterile 50 mL conical tubes and store at -20°C until needed.

F. QUALITY CONTROL

- Aliquot into smaller containers if to be used for multiple cases. Avoid multiple freeze-thaws when using pre-made, frozen aliquots.

G. INTERPRETATION

N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *M. cerebralis* Spores

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Brain Heart Infusion (BHI) Agar**	
SOP #: 803	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operation procedure (SOP) details the methods to prepare Brain Heart Infusion (BHI) agar plates.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment[1] and aseptic technique when preparing this media.

D. EQUIPMENT AND MATERIALS REQUIRED

1. BHI agar powder
2. dH_2O
3. Weigh boats
4. Scale
5. Glass bottles/flasks
6. Stir bar
7. Autoclave tape
8. Autoclave
9. Labeling tape
10. Sharpies

E. PROCEDURES

1. Prepare BHI agar per manufacturer instructions by suspending 52 g of power in 1 L distilled water.
2. Heat on hotplate with stir bar until dissolved.
3. Autoclave at 121°C for 15 minutes.
4. Remove from autoclave and cool to approximately 50°C. This can be done in a water bath set to 50°C if desired.
5. Pour plates into sterile Petri dishes as aseptically as possible and allow to cool to room temperature. Agar should be set before plates are stored in sleeve.

F. QUALITY CONTROL

- Follow aseptic technique, use sterile equipment when possible, and work in biosafety cabinet if available.

G. INTERPRETATION

Agar plates can be used to culture a variety of bacterial species.

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Carbonate/Bicarbonate Coating Buffer**	
SOP #: 804	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the method for preparing a carbonate/bicarbonate coating buffer for use in enzyme linked immunosorbent assays (ELISA).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution when measuring dry ingredients; wear a protective face mask[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Na_2CO_3
2. $NaHCO_3$
3. dH_2O
4. 1.0 L glass media bottle
5. Stir bar

E. PROCEDURES

1. Preparation:

	1 liter 1X
Na_2CO_3	1.59 g
$NaHCO_3$	2.59 g
dH_2O	1.0 L

2. Combine listed ingredients in a one liter bottle.
3. Mix on stir plate to dissolve.
4. Check and adjust pH to 9.6.

F. QUALITY CONTROL

N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 418, *Flavobacterium psychrophilum* Enzyme Linked Immunosorbent Assay (ELISA)

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Carbol Fuchsin Stain**	
SOP #: 805	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to prepare carbol fuchsin stain for use in pepsin-trypsin digestion (PTD) for recovery of *M. cerebralis* spores.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure proper personal protection[1] when handling carbol fuchsin.
- Carbol fuchsin is corrosive, can cause acute toxicity, skin irritation, eye irritation and respiratory sensitization. Wear gloves and face mask/shield.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Carbol fuchsin
2. 90% ethanol
3. dH_2O
4. Weigh boat
5. 0.45 µm filter
6. 5 mL syringes
7. Scale
8. Graduated cylinder
9. P-1000 pipette
10. P-1000 pipette tips

E. PROCEDURES

Carbol fuchsin	0.3 g
90% EtOH	500 µl
dH_2O	50 mL

1. Add 0.3 g carbol fuchsin to 500 µl of 90% EtOH.
2. Add 50 mL distilled H_2O.
3. Filter sterilize using 0.45 µm filter and 5 mL syringe.
4. Let solution stand for 30 minutes.

F. QUALITY CONTROL

N/A

ORIGINAL

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *M. cerebralis* Spores
3. SOP# 504, Staining and Reading Slides for The Detection of *M. cerebralis* Spores
4. SOP# 816, Methyl Blue Stain Preparation
5. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Cytophaga Agar**	
SOP #: 806	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedure to prepare Cytophaga agar for culturing *Flavobacterium* species.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Personal protective equipment should be worn[1].

EQUIPMENT AND MATERIALS REQUIRED

1. Tryptone (BD 211705)
1. Yeast extract (BD 90000-726)
2. Beef extract (HiMedia RM669-500g)
2. Sodium acetate
3. Agar (BD 214530)
4. Deionized water
5. Appropriate bottles with lids
6. Tinfoil
7. Stir bar
8. Petri plates (BD Falcon 351029)
9. Autoclave tape
10. Tobramycin (Sigma T4014-100 mg) rehydrated in 10 mL DI H_2O

D. PROCEDURES

1. Preparation of media:

	100 mL	500 mL	1000 mL
Tryptone	0.05 g	0.25 g	0.5 g
Yeast extract	0.05 g	0.25 g	0.5 g
Beef extract	0.02 g	0.10 g	0.2 g
Sodium acetate	0.02 g	0.10 g	0.2 g
Agar	1.00 g	5.00 g	10 g
DI H_2O	Qs to 100 mL	Qs to 500 mL	Qs to 1000 mL
Tobramycin	50 µL	250 µL	500 µL

- Mix all ingredients together in an appropriate size bottle using stir bar and stir plate.
- Adjust pH to between 7.1 and 7.3.
- Leaving lid slightly loose, cover entire top of bottle with tinfoil and label with autoclave tape.
- Autoclave at 121°C for 15 minutes.
- Allow media to cool to 55°C.

ORIGINAL

- If using Tobramycin add the appropriate amount and mix gently.
- Pour aliquots of approximately 20 mL into petri plates.
- Allow to cool until solid.
- Label plates with media type and label sleeve with date and initials.
- Invert plates and store plates with the media side up at 2ºC in media refrigerator.

2. Pouring plates:
   - If refrigerated, warm bottle of media to room temperature in water bath.
   - Autoclave at 121°C for 15 min, then cool to 56°C in water bath.
   - Preferably, under laminar flow hood in cell culture, aseptically pour aliquots into large petri plates.
   - Stack and let cool overnight, then label with media type, date, and initials.
   - Store with media side up.
3. Modified Cytophaga agar recipe:

	100mL
Tryptone	0.2g
Yeast Extract	0.05g
Beef Extract	0.02g
Sodium Acetate	0.02g
Calcium Chloride	0.02g
Agar	1.5g

   - pH media to 7.1.

E. QUALITY CONTROL

N/A

F. INTERPRETATION

- Media should not be cloudy or discolored. Check for sterility prior to use.

G. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

H. REVISION HISTORY

1. Original

I. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: 55% Dextrose Solution**	
SOP #: 807	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods to prepare a 55% dextrose solution for the use in a dextrose filtration procedure for the concentration of *M. cerebralis* spores after pepsin-trypsin digestion (PTD) of fresh tissues.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- The hot plate and glass flask will get hot, use caution when handling the hot plate and associated materials[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. dH_2O
2. 1 L glass bottle or 1 L flask
3. Stir bar
4. Stir plate
5. Aluminum foil
6. Dextrose solute
7. Weigh boats
8. Labeling tape
9. Sharpie
10. Autoclave
11. Autoclave tape

E. PROCEDURES

Dextrose	220 g
dH_2O	400 mL

1. Add approximately 200 mL of distilled water to 1 L volumetric flask, gently add stir bar, center on stir plate. Stir at a moderate speed and turn heat on low.
2. Weigh 220 g of dextrose solute into large weigh boats in increments of 100 g each. Add dextrose slowly to the flask ensuring the continual movement of the stir bar and to prevent scorching. When the entire amount of dextrose (220 g) has been dissolved, allow solution to return to room temperature.
3. Add remaining 200 mL of dH_2O to final volume of 400 mL and cover with aluminum foil.
4. Label with reagent name, date and initials.
5. Place autoclave tape on foil and autoclave flask for 15 minutes at 121°C.
6. Swirl to mix/place on stir plate after autoclaving.
7. Place in reagent refrigerator and store at 2-4°C.

ORIGINAL

F. QUALITY CONTROL

- Aliquot solution into smaller containers if to be used for multiple cases. Never use stock solution on bench top if needed to be used again for another case.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery Of *M. cerebralis* Spores
3. SOP# 504, Staining and Reading Slides for the Detection of *M. cerebralis* Spores

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Antibody Conjugate for *Renibacterium salmoninarum***	
SOP #: 808	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the method to prepare antibody conjugate used to observe *Renibacterium salmoninarum* in tissue with a fluorescent microscope.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate protective equipment when handling reagents[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Fluorescein-labeled, affinity purified antibody to *Renibacterium Salmoninarum* (BKD conjugate)
   - Kirkegaard and Perry (KPL) is the sole manufacturer of affinity purified, fluorescein-labeled antibodies to *Renibacterium salmoninarum*. An equivalent product can be used when available.
2. Molecular grade H_2O
3. Sterile pipettes
4. Sterile 50 mL centrifuge tube
5. Sterile amber microcentrifuge tubes

E. PROCEDURES

1. Rehydrate the Fluorescein Labeled Affinity Purified Antibody following manufacturer's instructions by adding 1 mL of molecular grade water to the vial.
2. Return the rubber stopper and lid to the vial and mix well.
3. Aliquot 100 µL into 10-500 µL amber microcentrifuge tubes and freeze, wrapped in tinfoil for future use.
4. Label with the name of the reagent, date, lot number from the vial, and initials.
8. Store at -80°C.

F. QUALITY CONTROL

- Check each antibody lot for fluorescence with a known positive tissue control.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 414, *Renibacterium salmoninarum* Direct Fluorescent Antibody Test

ORIGINAL

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Dulbecco Minimal Essential Culture Media (DMEM)**	
SOP #: 809	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to create a medium used for cell culture. Dulbecco Minimal Essential Culture Media (DMEM) 10 is used for splitting and expanding cell lines and DMEM 5 is used for maintaining cells after seeding into plates and inoculation with processed samples.

B. DEFINITIONS

1. DMEM 10: Dulbecco's Modified Eagle Media (with 10% FBS)
2. FBS: Fetal Bovine Serum
3. DMEM 5: Dulbecco's Modified Eagle Media (with 5% FBS)
4. 10x DMEM: Concentrated DMEM for media prep

C. SAFETY PRECAUTIONS

- Use appropriate protective equipment when handling reagents[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Endotoxin Free Water HyClone SH30529LS
2. DMEM : Dulbecco's Modified Eagle Media Sigma D1152
3. Fetal Bovine Serum HyClone SH300700
4. Bottle Top Filter 1 L 0.22uM Fisher 9761104
5. L-glutamine 200 mM HyClone SH300340

E. PROCEDURES

1. 10X DMEM:

Water (tissue culture grade, sterile)	1.0 L
DMEM powder	92 g

- o Add powdered media to sterile H_2O. Mix well and vacuum filter sterilize.
- o Label media type, date, and initial.
- o Store at 4°C.

2. DMEM 10:

	1.0 L
Water (tissue culture grade, sterile)	770.7 mL
10X DMEM	100 mL
1N NaOH	9.3 mL
l-glutamine (200mM)	20 mL
FBS	100 mL

- o Remove 229.3 mL water from sterile H_2O bottle and discard in order to accommodate liquid ingredients. Add remaining ingredients to sterile H_2O. Mix well and vacuum filter sterilize.

ORIGINAL

- Label media type, date, and initial.
- Store at 4°C.

3. DMEM 5:

	1.0 L
Water (tissue culture grade, sterile)	820.7 L
10X DMEM	100 mL
1N NaOH	9.33 mL
L-glutamine 200mM	20 mL
FBS	50 mL

- Remove 179.3 mL water from sterile H_2O bottle and discard in order to accommodate liquid ingredients. Add remaining ingredients to sterile H_2O. Mix well and vacuum filter sterilize.
- Label media type, date, and initial.
- Store at 4°C.

F. QUALITY CONTROL

- Media preparation is sensitive to contamination. Aseptic techniques should be utilized at all times.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 601, Cell Culture and Seeding Plates
3. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: ***Flavobacterium psychrophilum* Plate Coating Antigen for ELISA**	
SOP #: 810	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/20/2017

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the method for preparing the plate coating antigen for use in the *Flavobacterium psychrophilum* enzyme linked immunosorbent assay (ELISA)[1].

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. MAT broth
2. TYES agar plates
3. PBS
4. 1.0 L flask
5. Inoculation loops
6. Parafilm
7. Frozen *F. psychrophilum* 259-93 stock
8. Foam bottle closures
9. Aluminum foil
10. Gram slides
11. Stir bars
12. Stir plate
13. Incubator set to 15°C
14. Centrifuge
15. Vortexer

ORIGINAL

E. PROCEDURES

1. Plate preparation:
   - Prepare MAT broth[2] and TYES[3] plates according to lab protocols.
   - Place broth and plates in incubator at 15ºC.
2. Bacterial culture:
   - Retrieve frozen stock of *F. psychrophilum* (strain 259-93) from -80°C. Allow to partially thaw at room temperature.
   - Streak TYES plates with loop of partially thawed *F. psychrophilum* culture so as to obtain distinct separate colonies for isolation and testing.
   - Incubate at 15ºC for approximately 72 hours.
   - Select a distinct colony from the plate, Gram stain[4] and run a FL-43 DFAT[5] to confirm *F. psychrophilum*.

- Inoculate flask of 15°C MAT broth with a loop of *F. psychrophilum* (strain 259-93) from a 72 hour culture on TYES plates. Place foam stopper inside flask opening and cover with aluminum foil.
- Place on stir plate inside 15ºC incubator and stir very slowly for approximately 72 hours.
- At approximately 72 hours, fix broth culture with 0.04% formaldehyde.
- Let stir for 30 minutes, pellet cells at 5000 rpm for 20 minutes and then wash with PBS.
- Repeat two times.
- Resuspend cells in desired amount of PBS and sonicate.
- Streak on TYES to ensure cells are not viable.
- Determine protein concentration and dilute in PBS to a concentration of 1000 µg/mL.
- Store in 1.0 mL aliquots at -80ºC.

D. QUALITY CONTROL

- **N/A**

E. INTERPRETATION

N/A

F. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 418, *Flavobacterium psychrophilum* Enzyme Linked Immunosorbent Assay (ELISA)
2. SOP# 815, Maltose and Acetate Supplemented Tryptone Yeast Extract Salts (MAT) Broth
3. SOP# 838, Tryptone Yeast Extract Salts Agar (TYES)
4. SOP# 404, Gram Staining Procedure
5. SOP# 415, *Flavobacterium psychrophilum* Direct Fluorescent Antibody Test

G. REVISION HISTORY

1. Original

H. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: ***Flavobacterium psychrophilum* ELISA Plate Coating Antigen Solution**	
SOP #: 811	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the method for preparing a plate coating antigen solution for use with the *Flavobacterium psychrophilum* coating antigen prepared for the *Flavobacterium psychrophilum* enzyme linked immunosorbent assay (ELISA).[1]

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Carbonate/Bicarbonate Coating Buffer[2]
2. *Flavobacterium psychrophilum* Plate Coating Antigen For ELISA[3]
3. 250 mL beaker
4. Stir bar
5. Pipettor and tips

E. PROCEDURES

1. Preparation:

	100 mL
Coating antigen[3] (1000 µg/mL)	1.0 mL
Carb/bicarb buffer[2]	99.0 m

2. Combine listed ingredients in a beaker and mix on a stir plate.

D. QUALITY CONTROL

- N/A

E. INTERPRETATION

N/A

F. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 418, *Flavobacterium psychrophilum* Enzyme Linked Immunosorbent Assay (ELISA)
2. SOP# 804, Carbonate/Bicarbonate Coating Buffer
3. SOP# 810, *Flavobacterium psychrophilum* Plate Coating Antigen for ELISA

ORIGINAL

G. REVISION HISTORY

1. Original

H. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Giemsa Stain**	
SOP #: 812	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to stain blood smears and/or imprints for the presence of pathogens.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use appropriate personal protective equipment (PPE) when handling methanol and stain reagents[1].

A. EQUIPMENT AND MATERIALS REQUIRED

1. Methanol 100%
2. dH_2O
3. Giemsa Stain Prep (Sigma #GS500)
4. Slide dryer
5. Coplin jar

B. PROCEDURES

1. Dilute Giemsa stain 1:20 with deionzed water in Coplin jar.
2. Label jar with date stain solution was prepared.
3. Store in stain cabinet.

C. QUALITY CONTROL

- Make sure stain is within expiry and discard if precipitate has formed. It is advised to make fresh stain solution each time needed.

D. INTERPRETATION

Geimsa staining can be used on tissue imprints/smears[2] or on various other samples to look for bacteria or parasites. Nuclei will stain purple and cytoplasm will stain varying shades of blue to light pink. Basophils will stain dark blue and eosinophils will stain orange.

E. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 402, Giemsa Staining Of Smears And Imprints

F. REVISION HISTORY

1. Original

ORIGINAL

G. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Hank's Balanced Salt Solution (HBSS)**	
SOP #: 813	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to prepare Hank's Balanced Salt Solution (HBSS) media.

B. DEFINITIONS

1. HBSS: Hank's Balanced Salt Solution
2. P/S: Penicillin/Streptomycin solution

C. SAFETY PRECAUTIONS

- Use gloves when handling antibiotics[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Water (tissue culture grade, sterile) (HyClone SH30529.03)
2. 10X HBSS powder (HyClone SH30016.02)
3. Penicillin/Streptomycin (store frozen) (HyClone SV30010)
4. Amphotericin B (store frozen) (HyClone SV30078.01)
5. Sodium bicarbonate $NaHCO_3$ (HyClone SH30033.01)
6. Vacuum filter bottles

E. PROCEDURES

1. <u>10X HBSS:</u>

Water	1.0 L
HBSS (powder)	46.0 g

   - Decant 200 mL of water from a sterile H2O bottle and add HBSS powder. Mix well, q.s. to 1.0 L, and filter sterilize.
   - Label media type, date, and initial.
   - Store at 4°C.

2. <u>1X HBSS:</u>

Water	1.0 L
10X HBSS	100 mL
$NaHCO_3$	34.7 mL

   - Decant 134.7 mL of water from a sterile H2O bottle and add 10X HBSS and $NaHCO_3$. Mix well and filter sterilize.
   - Label media type, date, and initial.
   - Store at 4°C.

F. QUALITY CONTROL

- Media preparation is sensitive to contamination. Aseptic techniques should be utilized at all times.

ORIGINAL

A. INTERPRETATION

N/A

B. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 601, Cell Culture and Seeding Plates
3. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

C. REVISION HISTORY

1. Original

D. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **MacConkey Agar**	
SOP #: 814	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make this differential medium used to isolate and differentiate lactose-fermenting and lactose-nonfermenting Gram-negative enteric bacilli.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

A. EQUIPMENT AND MATERIALS REQUIRED

1. MacConkey Agar (BD 212123)
2. Stir bar
3. Media bottles with lids
4. Deionized water
5. Aluminum foil
6. Petri plates (BD Falcon 351029)
7. Autoclave tape

B. PROCEDURES

MacConkey agar	25 g
Deionized water	500 mL

1. Suspend 25 g of MacConkey Agar in 500 mL of deionized water and mix thoroughly in a 1.0 L glass media bottle with lid.
2. Leaving lid slightly loose, cover entire top of bottle with aluminum foil and label with autoclave tape.
3. Autoclave at 121°C for 15 minutes.
4. Allow media to cool to 55°C. This can be achieved by placing bottle into a preheated water bath if desired.

1. Pour aliquots of approximately 20 mL into petri plates.
2. Allow to cool until solid.
3. Label dishes with media type and label sleeve with date and initials.
4. Invert plates and store plates with the media side up at 2-4ºC in media refrigerator.

C. QUALITY CONTROL

- Use aseptic technique when pouring plates to limit the risk of contamination. Visually inspect each plate before use for any bacterial growth.

ORIGINAL

D. INTERPRETATION

Lactose fermenting bacteria will appear as pink colonies on MacConkey agar, as these species produce acid which lowers the pH of the agar. Lactose nonterminating bacteria will appear as white/cream colonies on MacConkey agar due to the production of ammonia, which raises the pH of the agar.

E. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

F. REVISION HISTORY

1. Original

G. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Maltose and Acetate Supplemented Tryptone Yeast Extract Salts (MAT) Broth**	
SOP #: 815	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to make maltose and acetate supplemented tryptone yeast extract salts (MAT) broth, a selective growth medium for *Flavobacterium psychrophilum*.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Tryptone (BD 211705)
2. Yeast extract (BD 90000-726)
3. $MgSO_4$
4. $CaCl_2$
5. Maltose
6. NaOAc (sodium acetate)
7. Stir bar
8. 2.5-3.0 L Fernbach flask
9. Deionized water
10. Tinfoil
11. Foam stopper
12. Autoclave tape
13. Autoclave

E. PROCEDURES

	1000 mL
Tryptone	4.0 g
Yeast extract	0.4 g
$MgSO_4$	0.5 g
$CaCl_4$	0.5 g
Deionized water	1000 mL
Maltose	10.0 g
NaOAc	0.2 g

1. Combine first 4 listed ingredients with 1000 mL of deionized water and mix thoroughly in a 3.0 L glass Fernbach flask.
2. Check and adjust pH to 7.1-7.3.

ORIGINAL

3. Add maltose and NaOAc and mix on a stir plate.
4. Insert foam stopper half way into the flask opening and then cover the entire top of bottle with aluminum foil and label with autoclave tape.
5. Autoclave at 121°C for 30 minutes.
6. Allow media to cool and then place at 2-4°C until ready for use.

F. QUALITY CONTROL

- Use aseptic technique when preparing media, limit the risk of contamination as much as possible. Visibly inspect the broth before use for any bacterial growth (i.e., turbidity).

G. INTERPRETATION

This media is used primarily for growing *F. psychrophilum* in preparation for challenging fish for experimental infections.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 416, *Flavobacterium psychrophilum* Bacterial Challenge

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Methyl Blue Stain**	
SOP #: 816	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods for preparation of methyl blue stain for use in pepsin-trypsin digestion (PTD) for recovery of *M. cerebralis* spores.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Ensure protection when handling methyl blue[1].
- Methyl blue can cause acute toxicity, skin irritation and eye irritation. Wear gloves and face mask/shield.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Methyl blue
2. dH_2O
3. 0.45 µm filter
4. 10 mL syringe
5. Scale
6. Graduated cylinder

E. PROCEDURES

Methyl blue	0.05 g
dH_2O	100 mL

1. Add 0.05 g methyl blue to 100 mL distilled water and mix thoroughly.
2. Filter sterilize using a 0.45 µm filter and 10 mL syringe.
3. Let solution stand for 30 minutes prior to use.

F. QUALITY CONTROL

N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *M. cerebralis* Spores
3. SOP# 504, Staining and Reading Slides for the Detection of *M. cerebralis* Spores
4. SOP# 805, Carbol Fuchsin Stain Preparation

ORIGINAL

5. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **MS-222 (Tricaine Methanesulfonate)**	
SOP #: 817	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods to prepare tricaine methanesulfonate (MS-222) for use in anesthetizing or euthanizing fish.

B. DEFINITIONS

1. Tricaine methanesulfonate: MS-222

C. SAFETY PRECAUTIONS

- Be aware of a 21 day withdrawal time. Do not use on fish that may be stocked or consumed for food within a 21 day window of using MS-222.
- Wear protective clothing, gloves, and safety glasses[1] when handling MS-222 powder. Limit the risk of inhalation.
- Work inside a fume hood to prepare a concentrated stock solution.
- Wear gloves to handle water medicated with and animals exposed to MS-222.

D. EQUIPMENT AND MATERIALS REQUIRED

1. MS-222 powder (Tricaine-S, Western Chemical, Inc.)
2. dH_2O
3. Sodium bicarbonate
4. Flask/bottle
5. Stir bar
6. Stir plate
7. Weigh boat
8. Amber colored 50 mL conical tubes

E. PROCEDURES

MS-222	100 g
Sodium bicarbonate	400 g
dH_2O	1 L

1. Preparation of concentrated solution:
   - Weigh out 100 g of MS-222 powder and add to 1 L dH_2O.
   - Weigh out 400 g of sodium bicarbonate and add to solution.
     - Sodium bicarbonate is added at a1:4 ratio to buffer acidifying effects of MS-222 on water.
   - Add a stir bar and let mix on stir plate.
   - After the solution has thoroughly been mixed, aliquot into 50 mL amber-colored conical tubes.
     - MS-222 is light sensitive and must be stored in a manner protecting it from light, such as in amber colored tubes.
   - Store at 4°C until ready for use.

ORIGINAL

2. Use of prepared concentrated solution:
   - Add concentrated solution to fresh water in appropriate volumes for desired outcome
     - 1 mL concentrated solution/1 L of fresh water = 100 mg/L
     - 15 mL concentrated solution/5 gallons of fresh water = anesthetize fish
     - 15 mL concentrated solution/2 gallons of fresh water = euthanize fish
3. Preparing MS-222 for single use:
   - Calculate out appropriate amounts of each reagent to be used based on volume of fresh water.
     - 1 g MS-222/ 4 L fresh water (for euthanasia)
     - 0.4 g MS-222/ 4 L fresh water (for anesthesia)
     - 4 g sodium bicarbonate/ 1 g MS-222
   - Measure out appropriate amounts of reagents and add to fresh water.

F. QUALITY CONTROL

N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Mueller Hinton Agar**	
SOP #: 818	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make Mueller Hinton Agar which is a general media used for growing bacteria and for antibiotic disc sensitivity assays.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Mueller Hinton Agar (BD 211443)
2. Stir bar
3. Media bottles (or flasks) with lids
4. Deionized water
5. Aluminum foil
6. Petri plates (BD Falcon 351029)
7. Autoclave tape

E. PROCEDURES

Mueller Hinton agar 11 g
Deionized water 500 mL

1. Suspend 11 g of Mueller Hinton Agar powder in 500 mL of deionized water and mix thoroughly in a 1.0 L glass media bottle with lid.
2. Leaving lid slightly loose, cover entire top of bottle with aluminum foil and label with autoclave tape.
3. Autoclave at 121°C for 15 minutes.
4. Allow media to cool to 55°C. This can be achieved by placing bottle into a preheated water bath if desired.

1. Pour aliquots of approximately 20 mL into petri plates.
2. Allow to cool until solid.
3. Label dishes with media type and label sleeve with date and initials.
4. Invert plates and store plates with the media side up at 2-4ºC in media refrigerator.

ORIGINAL

F. QUALITY CONTROL

- Use aseptic technique when pouring plates to limit the risk of contamination. Visually inspect each plate before use for any bacterial growth.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: Myxospore Re-suspension Media**	
SOP #: 819	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE
The purpose of this standard operating procedure (SOP) is to describe the process used to make myxospore re-suspension media needed to mount samples for slide reading.

B. DEFINITIONS
1. Phosphate buffered saline (PBS)
2. Ethanol (EtOH)

C. SAFETY PRECAUTIONS
- Use appropriate personal protective equipment when working in the lab[1].

D. EQUIPMENT AND MATERIALS REQUIRED
1. Sterile PBS
2. 100% EtOH
3. 50 mL tubes

E. PROCEDURES
1. Add 5 mL EtOH to 45 mL sterile PBS in a 50 mL tube and rock to mix.
   - Re-suspension media may be stored in fridge or freezer and used as needed.

F. QUALITY CONTROL
- If re-suspension media is visually contaminated, new media must be made.

G. INTERPRETATION
Re-suspension media allows for better mounting of samples onto slides for final staining and reading.

H. ASSOCIATED DOCUMENTS/REFERENCES
1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for the Recovery of *Myxobolus cerebralis* Spores
3. SOP# 503, Glucose/Sucrose Filtration For *Myxobolus cerebralis* Spore Concentration
4. SOP# 504, Staining and Reading Slides for the Detection of *Myxobolus cerebralis* Spores

I. REVISION HISTORY
1. Original

J. APPENDICES
- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Enriched Ordahls Agar**	
SOP #: 820	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedure required to prepare Enriched Ordahls Agar for culturing *Flavobacterium* species.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Tryptone
2. Yeast extract
3. Beef extract
4. Sodium acetate
5. Agar
6. Deionized water
7. Appropriate bottles with lids
8. Tinfoil
9. Stir bar
10. Petri plates
11. Autoclave tape
12. Tobramycin rehydrated in 10 mL DI H2O

E. PROCEDURES

1. Preparation of media:

	100 mL	500 mL	1000 mL
Tryptone	0.50 g	2.50 g	5.0 g
Yeast extract	0.05 g	0.25 g	0.5 g
Beef extract	0.02 g	0.10 g	0.2 g
Sodium acetate	0.02 g	0.10 g	0.2 g
Agar	1.00 g	5.00 g	10 g
DI H_2O	Qs to 100 mL	Qs to 500 mL	Qs to 1000 mL
Tobramycin	50 µL 500 µL		250 µL

- Mix all ingredients together in an appropriate size bottle using stir bar and stir plate.
- Adjust pH to between 7.1 and 7.3.
- Leaving lid slightly loose, cover entire top of bottle with tinfoil and label with autoclave tape.
- Autoclave at 121°C for 15 minutes.

- Allow media to cool to 55°C.
- If using Tobramycin, add the appropriate amount and mix gently.
- Pour aliquots of approximately 20 mL into petri plates.
- Allow to cool until solid.
- Label plates with media type and label sleeve with date and initials.
- Invert plates and store plates with the media side up at 2ºC in media refrigerator.

F. QUALITY CONTROL

- Visually confirm sterility of media before use.

G. INTERPRETATION

Flavobacterium colonies will appear yellow in color.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Phosphate Buffered Saline (PBS)**	
SOP #: 821	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the method for preparing Phosphate Buffered Saline (PBS).

B. DEFINITIONS

N/A

C. SAFETY PRECAUTIONS

- Use caution when measuring dry ingredients.
- Wear a protective face mask.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sodium chloride (NaCl)
2. Potassium chloride (KCl)
3. Sodium phosphate dibasic (Na_2HPO_4)
4. Sodium phosphate monobasic (NaH_2PO_4)
5. 1.0 L glass media bottle
6. Stir bar
7. PBS tablet or packet

E. PROCEDURES

1. Preparation for non-technical use:

	1 liter 1X	1 liter 10X
NaCl	8.0 g	80.0 g
Na_2HPo4	1.14 g	11.4 g
NaH_2PO_4	0.26 g	2.62 g
KCl	0.2 g	2.0 g
DH_2O to 1 L		

   - Combine listed ingredients in a one liter bottle.
   - Add dH_2O to achieve1.0 L.
   - Mix on stir plate to dissolve.
   - Autoclave if desired.
2. Sterile PBS preparation:
   - Dispense tablet or packet in a one liter bottle.
   - Add dH_2O to achieve1.0 L.
   - Mix on stir plate to dissolve.
   - Autoclave if desired

F. QUALITY CONTROL

N/A

ORIGINAL

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 418, *Flavobacterium psychrophilum* Enzyme Linked Immunosorbent Assay (ELISA)

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: PBS + Tween 20**	
SOP #: 822	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedure to prepare a wash solution for the *Flavobacterium psychrophilum* enzyme linked immunosorbent assay (ELISA).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Phosphate buffered saline (PBS)
2. Tween 20
3. 5.0 L glass media bottle
4. Pipetter
5. Pipette tips

E. PROCEDURES

Tween 20	2.5 mL
PBS	5.0 L

1. Combine 2.5 mL Tween 20 with 5.0 L PBS in a large bottle.
2. Shake vigorously by hand to mix.

F. QUALITY CONTROL

N/A

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 418, Flavobacterium psychrophilum Enzyme Linked Immunosorbent Assay (ELISA)
2. SOP# 821, Phosphate Buffered Saline
3. SOP# 810, Flavobacterium psychrophilum Plate Coating Antigen for ELISA

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: PBS-Tween 20 + 0.1% Non-Fat Milk (PBS-T+NFM)**	
SOP #: 823	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE
The purpose of this standard operating procedure (SOP) is to describe the method for preparing PBS-T + NFM solution for the *Flavobacterium psychrophilum* ELISA.

B. DEFINITIONS
1. N/A

C. SAFETY PRECAUTIONS
- N/A

D. EQUIPMENT AND MATERIALS REQUIRED
1. PBS-Tween
2. Non-fat milk powder (NFM)
3. 250 mL glass beaker
4. Stir bar

E. PROCEDURES
1. To prepare 100 mL of the solution, combine 100 mL PBS-Tween and 0.1 g NFM powder.
2. Add a stir bar, place on the stir plate and mix until totally dissolved.

F. QUALITY CONTROL
- Use only laboratory grade NFM powder.

G. INTERPRETATION
N/A

H. ASSOCIATED DOCUMENTS/REFERENCES
1. SOP# 418, *Flavobacterium psychrophilum* Enzyme Linked Immunosorbent Assay (ELISA)
2. SOP# 821, Phosphate Buffered Saline
3. SOP# 810, *Flavobacterium psychrophilum* Plate Coating Antigen for ELISA

I. REVISION HISTORY
1. Original

J. APPENDICES
- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title**: PBS and 0.5% Non-Fat Dry Milk**	
SOP #: 824	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make 0.5% Non-Fat Dry Milk + PBS solution used for DFAT staining for *Flavobacterium psychrophilum* sample screening.

B. DEFINITIONS

1. PBS: Phosphate buffered saline
2. NFDM: Non-fat dry milk

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working in the lab.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Non-fat dry milk
2. PBS (pre-made)
3. 15 mL tubes

E. PROCEDURES

1. NDFM+PBS Stock solution:
   - Hydrate 0.75 g of NFDM in 15 mL PBS for a concentration of 0.5% w/v. Solution should be at pH 7.2.

F. QUALITY CONTROL

- Solution must be made fresh if contamination is visible.

G. INTERPRETATION

Solution must be re-made if positive control cannot be visualized.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 415, *Flavobacterium psychrophilum* Direct Fluorescent Antibody Test

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Pepsin Solution**	
SOP #: 825	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operation procedure (SOP) describes the methods to prepare pepsin solution for use in the pepsin-trypsin digestion[1] (PTD) of fresh tissues for the recovery of *M. cerebralis* spores. Pepsin is used as the primary enzymatic reagent in the PTD assay as it is used to digest fresh bone and cartilage.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution and wear appropriate personal protective equipment[2] when handling chemicals, particularly hydrochloric acid (HCl).

D. EQUIPMENT AND MATERIALS REQUIRED

1. Pepsin
2. Concentrated HCl
3. 4 L flask
4. dH_2O
5. 1000 mL plastic bottles
6. Stir bar
7. Scale
8. Stir plate

E. PROCEDURES

	2000 mL	4000 mL
Pepsin	10 g	20 g
Concentrated HCl	10 mL	20 mL
dH_2O	1990 mL	3980 mL

1. Place a stir bar in a 4 L flask, add appropriate volume of concentrated HCl (carefully), center on sitr plate and stir at moderate speed.
2. Weigh out appropriate amount of powdered pepsin and slowly add to HCl.
3. Carefully add the appropriate volume of dH_2O.
4. Stir until pepsin is fully dissolved and solution is homogenous.
5. Cover with aluminum foil and store at 4°C until use.

F. QUALITY CONTROL

- Aliquot solution into smaller containers if to be used for multiple cases. Never use stock solution on bench top if needed to be used again for another case.

G. INTERPRETATION

ORIGINAL

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *M. cerebralis* Spores
2. SOP# 201, Personal Protective Equipment
3. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Antimicrobials for SKDM-2**	
SOP #: 826	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to prepare antimicrobials for use in selective Kidney Disease Media-2[1] (SKDM-2; Austin et al. 1983) which is used to culture *Renibacterium salmoninarum*.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Wear appropriate protective equipment[2].
- Do not tighten caps on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Cycloheximide
2. D-cycloserine
3. Polymyxin B sulfate
4. Oxolinic acid
5. dH_2O
6. Weigh boats
7. Scale
8. 0.45 µm filters
9. 5 mL syringes
10. 1 N NaOH
11. 5 mL tubes

E. PROCEDURES

1. Preparation of Cycloheximide (0.0125 g/mL):
   - Measure 1.2 g of cycloheximide.
   - Measure 96 mL dH_2O.
   - Mix water and cycloheximide.
   - Filter sterilize using 0.45 µm filter and 5 mL syringe.
   - Aliquot 4 mL solution into 5 mL tubes.
   - Store at -20ºC.
2. Preparation of D-cycloserine (0.0125 g/mL):
   - Measure 0.3 g of D-cycloserine.
   - Measure 24 mL dH_2O.
   - Mix water and D-cycloserine.
   - Filter sterilize using 0.45 µm filter and 5 mL syringe.
   - Aliquot1 mL solution into 1.5 mL microcentrifuge tubes.
   - Store at -20ºC.

3. Polymyxin B sulfate (0.0125 g/mL):
    - Measure 0.3 g of polymyxin B sulfate.
    - Measure 24 mL dH_2O.
    - Mix water and polymyxin B sulfate.
    - Filter sterilize using 0.45 µm filter and 5 mL syringe.
    - Aliquot 2 mL solution into 2 mL microcentrifuge tubes.
    - Store at -20ºC.
4. Oxolinic acid (0.0025 g/mL):
    - Measure 0.06 g of oxolinic acid.
    - Measure 24 mL 5% NaOH.
    - Mix NaOH and oxolinic acid.
    - Filter sterilize using 0.45 µm filter and 5 mL syringe.
    - Aliquot 1 mL solution into 1.5 mL microcentrifuge tubes.
    - Store at -20ºC.

F. QUALITY CONTROL

- Use aseptic technique when preparing reagents to limit the risk of contamination.
- Keep aliquots frozen when not in use.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 829, Selective Kidney Disease Media-2 (SKDM-2)
2. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **Rhodamine B**	
SOP #: 827	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make rhodamine b solution used for DFAT staining of BKD sample screening.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use proper personal protective equipment[1] when working in the lab[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Molecular grade water
2. Rhodamine B
3. 15 mL tubes

E. PROCEDURES

1. Rhodamine B stock solution
   - Hydrate rhodamine B powder in reagent quality water to 10 mg/mL.
2. Preparation of working solution and stain
   - Dilute 10 mg/mL stock solution to 1 mg/mL. This is the working concentration.
     - To prepare 5 mL of fluorescein stain, Mix 4.9 mL PBS, 50 µL of labeled antibody[2] and 50 µl of the 1 mg/mL rhodamine working stock.
     - Vortex and filter through a 0.45 µm filter with a 10 mL syringe.
     - Stain is ready to use and is stored wrapped in foil or an amber tube.

F. QUALITY CONTROL

Stain must be filtered before use. If debris is noted, new stain must be made.

G. INTERPRETATION

Stain must be re-made if positive control cannot be visualized.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 414, *Renibacterium salmoninarum* Direct Fluorescent Antibody Test

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Rinaldini's Solution**	
SOP #: 828	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods for the preparation of Rinaldini's solution for the use in pepsin-trypsin digestion (PTD) for recovery of *M. cerebralis* spores.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution and wear appropriate PPE when handling chemicals[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Sodium chloride (NaCl)
2. Potassium chloride (KCl)
3. Sodium citrate dihydrate ($C_6H_5Na_3O_7*_2H_2O$)
4. Sodium phosphate monobasic monohydrate ($(NaH_2Po_4)*H_2O$)
5. Sodium bicarbonate ($NaHCo_3$)
6. Glucose ($C_6H_{12}O_6$)
7. Phenol red solution (pre-prepared solution Sigma Cat# P0290-100ML)
8. dH_2O
9. 2 L flask
10. Weigh boat
11. Stir bar
12. Stir plate
13. Graduated cylinder
1. 250 mL reagent bottles

B. PROCEDURES

<u>Total volume 2 L</u>

Sodium chloride (NaCl)	80.0 g
Potassium chloride (KCl)	2.0 g
Sodium citrate dihydrate ($C_6H_5Na_3O_7*_2H_2O$)	10.0 g
Sodium phosphate monobasic monohydrate ($(NaH_2Po_4)* H_2O$)	0.5 g
Sodium bicarbonate ($NaHCo_3$)	10.0 g
Glucose ($C_6H_{12}O_6$)	10.0 g
Phenol red solution	40 mL
dH_2O	1960 mL

1. Gently add stir bar to 2 L flask, add 1000 mL dH_2O and center on stir plate. Stir at moderate speed.
2. Measure out 960 mL dH_2O in graduated cylinder. Do not add to flask yet.

ORIGINAL

3. Weigh the required amount of each chemical (see list above) into a clean weigh boat. Transfer each chemical to the flask. Rinse weigh boat at end using dH_2O from graduated cylinder filled with 960 mL to remove residue and pour into flask.
4. Pour remaining dH_2O from graduated cylinder into flask.
5. Measure and add 40 mL phenol red solution.
6. Allow solution to stir until chemicals are completely dissolved.
7. Using a graduated cylinder, dispense reagent in to 200 mL aliquots using 250 mL reagent bottles. (10 total bottles will be needed for 2 L of prepared solution).
8. With laboratory labeling tape (or pre-made labels), label each bottle with reagent name, date and initials.
9. Freeze in designated reagent freezer in laboratory.

F. QUALITY CONTROL

Only remove and thaw bottles needed for the amount of trypsin you are preparing, this helps to reduce the risk of contaminating stock solutions.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery Of *M. cerebralis* Spores
3. SOP# 835, Trypsin Solutions
4. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Selective Kidney Disease Medium-2 (SKDM-2)**	
SOP #: 829	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make selective Kidney Disease Medium-2 (SKDM-2; Austin et al. 1983), which is used for the selective isolation of *Renibacterium salmoninarum*.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten caps on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. dH_2O
2. Stir bar
3. Stir plate
4. Glass bottle/flasks
5. Aluminum foil
6. Autoclave tape
7. Autoclave
8. Weigh boats
9. Scale
10. Peptone
11. Yeast extract
12. L-Cysteine HCl
13. Agar
14. NaOH or HCl to pH
15. Heat inactivated fetal bovine serum (FBS) or calf serum
16. Cycloheximide (1.2 g in 96 mL dH_2O)
17. D-cycloserine (0.3 g in 24 mL dH_2O)
18. Polymyxin B sulfate (0.3 g in 24 mL dH_2O)

E. PROCEDURES

	1000 mL	100 mL
Peptone	10 g	1.0 g
Yeast extract	0.5 g	0.05 g
L-Cysteine HCI	1.0 g	0.1 g
dH_2O	892 mL	89.2 mL
Agar	15 g	1.5 g

ORIGINAL

1. Add a stir bar to an appropriate bottle/flask and mix the first four listed ingredients on a stir plate until well mixed.
2. Check and adjust pH to 6.5 with 1 N NaOH or HCl.
3. Add agar and mix briefly.
4. Autoclave at 121ºC for 15 minutes and cool to 50-55ºC.
5. Just prior to pouring plates, the remaining ingredients must be added aseptically:

	1000 mL	100 mL
Heat inactivated FBS	100 mL	10 mL
Cycloheximide (0.0125 g/mL)*	4 mL	400 µL
D-cycloserine (0.0125 g/mL)*	1 mL	100 µL
Polymyxin B sulfate (0.0125 g/mL)*	2 mL	200 µL
Oxolinic Acid (0.0025 g/mL)*	1 mL	100 µL

*Aliquots kept frozen at -20ºC.

6. Mix solution gently to avoid formation of bubbles.
7. Pour aliquots of approximately 20 mL into petri dishes.
8. Allow to cool until solid.
9. Label dishes with media type and label sleeve with date and initials.
10. Invert plates and store plates with the media side up at 2-4°C in media refrigerator.

F. QUALITY CONTROL

Use aseptic technique when pouring plates to limit the risk of contamination. Visually inspect each plate before use for any bacterial growth.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 826, Preparation of Antimicrobials for KDM

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Title: **0.5N Sodium Hydroxide (NaOH) Solution**	
SOP #: 830	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make a 0.5 N solution of sodium hydroxide (NaOH) for use in the pepsin-trypsin digestion of heads and/or as a solution to adjust pH levels of media.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- THIS MATERIAL IS EXTREMELY CORROSIVE! Proper personal protective equipment[1] MUST be worn when working with this chemical.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Deionized H_2O
2. NaOH (Baker# 3708-05)
3. Glass volumetric bottle or flask

E. PROCEDURES

NaOH	20 g
DH2O	500 mL

1. Using gloves, lab coat and safety goggles, dissolve 20 g NaOH in 500 mL DH_2O and cool to room temperature
2. QS to 1 L

F. QUALITY CONTROL

The pH of this solution should be ≥14. Consult SDS when handling.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **53% Sucrose Solution**	
SOP #: 831	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating Procedure (SOP) describes the methods to prepare a 53% sucrose solution for the use in a sucrose filtration procedure for the concentration of *M. cerebralis* spores after pepsin-trypsin digestion (PTD) of fresh tissues.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- The hot plate and glass flask will get hot, use caution[1] when handling the hot plate and associated materials.

D. EQUIPMENT AND MATERIALS REQUIRED

1. dH_2O
2. 1 L flask (or glass bottle)
3. Stir bar
4. Stir plate
5. Aluminum foil
6. Sucrose solute
7. Weigh boats
8. Labeling tape
9. Sharpie
10. Autoclave
11. Autoclave tape

E. PROCEDURES

Sucrose solute	265 g
dH_2O	500 mL

1. Add approximately 250 mL of distilled water to 1 L volumetric flask (or bottle), gently add stir bar, center on stir plate. Stir at a moderate speed and turn heat on low.
2. Weigh 265 g of sucrose solute into large weigh boats in increments of 100 g each. Add sucrose slowly to the flask ensuring the continual movement of the stir bar and to prevent scorching. When the entire amount of sucrose (265 g) has been dissolved, allow solution to return to room temperature.
3. Add remaining 250 mL dH_2O to final volume of 500 mL and cover with aluminum foil (or cap).
4. Label with reagent name, date and initials and place at 2-4°C.

ORIGINAL

F. QUALITY CONTROL

- Aliquot solution into smaller containers if to be used for multiple cases. Never use stock solution on bench top if needed to be used again for another case.
- Sucrose solution will not keep for very long, so ensure integrity of reagent before using any pre-made solution. It's recommended to make fresh solution each time needed (or autoclave solution prior to long term storage).

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *M. cerebralis* Spores
3. SOP# 504, Staining and Reading Slides for the Detection of *M. cerebralis* Spores

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **1X Tris Borate EDTA (TBE) Buffer**	
SOP #: 832	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Wade Cavender	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

- **PURPOSE**
  The purpose of this standard operating procedure (SOP) is to describe the methods of preparing tris borate EDTA (TBE) buffer. This buffer is used for making agarose gels, and also in the gel apparatus during gel electrophoresis.

- **DEFINITIONS**
  1. N/A

- **SAFETY PRECAUTIONS**
  - Wear gloves[1] when preparing materials used in this SOP.

D. EQUIPMENT AND MATERIALS REQUIRED
1. 10X TBE
2. dH_2O
3. Graduated cylinder
4. Large Nalgene bottle

E. PROCEDURES
1. Add 100 mL 10X TBE to 900 mL dH2O to make 1X TBE buffer.
2. Label with date and initials and store at room temperature.

F. QUALITY CONTROL
N/A

G. INTERPRETATION
N/A

H. ASSOCIATED DOCUMENTS/REFERENCES
1. SOP# 201, Personal Protective Equipment
2. SOP# 718, Gel Electrophoresis And Visualization

I. REVISION HISTORY
1. Original

J. APPENDICES
- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Tobramycin**	
SOP #: 833	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

ORIGINAL

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to make tobramycin solution for addition to selective tryptone yeast extract salts (TYES) agar, a selective medium for the isolation of *Flavobacterium* species.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Tobramycin (Sigma T4014-100 mg)
2. Stir bar
3. Media bottles with lids
4. Deionized water

E. PROCEDURES

dH_2O	10 mL
Tobramycin	100 mg

1. Add 10 mL dH_20 to 100 mg Tobramycin and mix thoroughly. Solution can be mixed in glass bottle provided or in a separate clean vessel with stir bar.

F. QUALITY CONTROL

- Use aseptic technique when making media to limit the risk of contamination as much as possible.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 838, Tryptone Yeast Extract Salts Agar, TYES

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Transfer Media**	
SOP #: 834	Date Initiated:3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Chris Heck	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to prepare transfer media (TM) with antibiotic for kidney/spleen samples.

B. DEFINITIONS

1. 10X HBSS: Hanks Balanced Salt Solution
2. TM: transfer media
3. P/S: Penicillin/Streptomycin solution

C. SAFETY PRECAUTIONS

- Use gloves when handling antibiotics[1].

D. EQUIPMENT AND MATERIALS REQUIRED

1. Water (tissue culture grade, sterile) (HyClone SH30529.03)
2. 10X HBSS powder (HyClone SH30016.02)
3. Penicillin/Streptomycin (store frozen) (HyClone SV30010)
4. Amphotericin B (store frozen) (HyClone SV30078.01)
5. Sodium bicarbonate $NaHCO_3$ (HyClone SH30033.01)
6. Vacuum filter bottles

E. PROCEDURES

	500 mL
Water	287 mL
10X HBSS	50 mL
$NaHCO_3$	2.5 mL
P/S	80 mL
Amphotericin B	80 mL

1. Decant 435 mL water from sterile H_2O bottle and add HBSS, P/S, Amphotericin and $NaHCO_3$. Mix well, q.s. to 500 mL and filter sterilize.
2. Label media type, date, and initial.
3. Store at 4°C.

F. QUALITY CONTROL

- Media preparation is sensitive to contamination. Aseptic techniques should be utilized at all times.

A. INTERPRETATION

N/A

B. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 813, Hanks Balanced Salts Solution
3. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

C. REVISION HISTORY

1. Original

D. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Trypsin Solutions**	
SOP #: 835	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

This standard operating procedure (SOP) describes the methods to prepare trypsin solution for use in the pepsin-trypsin digestion (PTD) of fresh tissues for the recovery of *M. cerebralis* spores, as well trypsin used in cell culture. Trypsin is used as the secondary enzymatic reagent in the PTD assay as it is used to further digest fresh bone and cartilage.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Use caution and wear appropriate personal protective equipment[1] when handling chemicals.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Rinaldini's solution[2]
2. dH_2O
3. Trypsin powdered (parasitology)
4. Trypsin solution (virology)
5. 1 L flask
6. Graduated cylinder
7. Weigh boat
8. Scale
9. Stir bar
10. Stir plate

E. PROCEDURES

For Parasitology:

	350 mL (enough for one pooled case)	700 mL
Rinaldini's solution	100 mL	200 mL
Trypsin powder	0.875 g	1.75 g
dH_2O	250 mL	500 mL

1. Prepare or retrieve Rinaldini's solution, and transfer 100 mL to 0.5 L flask containing a stir bar. Rinse Rinaldini's solution reagent bottle with dH_2O and add to flask.
2. Weigh out 0.875 g of powered trypsin to the flask. Rinse weigh boat with dH_2O and add water to flask.
3. QS solution to 250 mL with dH_2O.
4. Place on stir plate and stir thoroughly.
5. Use solution with 3 hours of preparing.

ORIGINAL

For Virology:

1. Aliquot 6 mL of prepared trypsin (liquid) into 15 mL tubes and freeze. This will be used when seeding new plates or flasks[3].

F. QUALITY CONTROL

- Aliquot solution into smaller containers if to be used for multiple cases.
- Never use stock solution on bench top if needed to be used again for another case.

G. INTERPRETATION

Any solution that is contaminated or past expiration should not be used. If digestion is not achieved in PTD or cells do not separate when seeding plates, repeat process with new stock.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 201, Personal Protective Equipment
2. SOP# 828, Rinaldini's Solution Preparation
3. SOP# 601, Cell Culture and Seeding Plates
4. SOP# 502, Pepsin-Trypsin Digestion (PTD) for Recovery of *M. cerebralis* Spores
5. AFS-FHS (American Fisheries Society-Fish Health Section). FHS bluebook: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, most recent edition. Accessible at: http:afs-fhs.org/bluebook/bluebook-index.php.

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Trypticase Soy Broth (TSB)**	
SOP #: 836	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make Trypticase Soy Broth (TSB), a general broth media used for growing bacteria.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Trypticase Soy Broth (TSB) media (BD 211768)
2. Stir bar
3. Media bottles with lids
4. Deionized water
5. Aluminum foil
6. Autoclave tape

E. PROCEDURES

Trypticase soy broth	15 g
Deionized water	500 mL

1. Suspend 15 g of TSB powder in 500 mL of deionized water and mix thoroughly in a 1.0 L glass media bottle with lid.
2. Leaving lid slightly loose, cover entire top of bottle with aluminum foil and label with autoclave tape.
3. Autoclave at 121°C for 15 minutes.
4. Allow media to cool and label bottles with media type, date and initials.
5. Store in media refrigerator at 2-4ºC.

F. QUALITY CONTROL

- Use aseptic technique to limit the opportunity for contamination of broth.
- If broth is turbid (i.e., bacterial growth) do not use.

G. INTERPRETATION

N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Trypticase Soy Agar (TSA)**	
SOP #: 837	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to make Trypticase Soya Agar (TSA) a general agar media used for growing bacteria.

B. DEFINITIONS

1. N/A

C. SAFEY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Trypticase Soy Agar (TSA) (BD 211043)
2. Stir bar
3. Media bottles with lids
4. Deionized water
5. Aluminum foil
6. Petri plates (BD Falcon 351029)
7. Autoclave tape

E. PROCEDURE

Trypticase soy agar	20 g
Deionized water	500 mL

1. Suspend 20 g of TSA powder in 500 mL of deionized water and mix thoroughly in a 1.0 L glass media bottle with lid.
2. Leaving lid slightly loose, cover entire top of bottle with tinfoil and label with autoclave tape.
3. Autoclave at 121°C for 15 minutes.
4. Allow media to cool to approximately 50-55°C. This can be achieved by placing bottle into a preheated water bath if desired.
1. Pour aliquots of approximately 20 mL into petri plates.
2. Allow to cool until solid.
3. Label dishes with media type and label sleeve with date and initials.
4. Invert plates and store plates with the media side up at 2-4ºC in media refrigerator.

ORIGINAL

F. QUALITY CONTROL

- Use aseptic technique when pouring plates to limit the risk of contamination.
- Visually inspect each plate before use for any bacterial growth.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Tryptone Yeast Extract Salts Agar (TYES)**	
SOP #: 838	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Cristi Swan	Date Last Reviewed: 3/30/2017
Laboratory Section: Media	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to make tryptone yeast extract salts (TYES) agar, a selective medium for the isolation of *Flavobacterium* species. This particular recipe can be adjusted to include tobramycin which helps improve the selectivity of the medium.

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Do not completely tighten lids on media bottles prior to autoclaving to avoid explosion hazards.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Tryptone (BD211705)
2. Yeast extract (BD 90000-726)
3. $MgSO_4$
4. $CaCl_2$
5. Agar (BD214530)
6. Tobramycin (Sigma T4014-100 mg) rehydrated in 10 mL DI H_2O Stir bar
7. Media bottles with lids
8. Deionized water
9. Aluminum foil
10. Petri plates (BD Falcon 351029)
11. Autoclave tape
12. Autoclave

E. PROCEDURES

Ingredient	Amount
Tryptone	2.0 g
Yeast extract	0.2 g
$MgSO_4$	0.25 g
$CaCl_2$	0.25 g
Deionized water	500 mL
Agar	5.0 g
Tobramycin (if using)	250 µL

1. Combine first 4 listed ingredients with 500 mL of deionized water and mix thoroughly in a 1.0 L glass media bottle with lid.
2. Check and adjust pH to 7.1-7.3.
3. Add 5.0 g of agar and mix briefly on stir plate. Agar will not completely dissolve at room temperature.

ORIGINAL

4. Leaving lid slightly loose, cover entire top of bottle with aluminum foil and label with autoclave tape.
5. Autoclave at 121°C for 30 minutes.
6. Allow media to cool to approximately 50-55°C. This can be achieved by placing bottle into a preheated water bath if desired.
7. If using Tobramycin, add 250 µL to achieve a 5 µg/mL solution. Mix gently.
8. Pour aliquots of approximately 20 mL into petri plates.
9. Allow to cool until solid.
10. Label dishes with media type and label sleeve with date and initials.
11. Invert plates and store plates with the media side up at 2-4ºC in media refrigerator.

F. QUALITY CONTROL

- Use aseptic technique when pouring plates; limit the risk of contamination as much as possible.
- Visually inspect each plate before use for any bacterial growth.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 833, Tobramycin

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

Instrumentation 900

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **-80°C Freezers**	
SOP #: 901	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of general care and maintenance of the -80° freezers.

B. EQUIPMENT INFROMATION

Large freezer in main lab
Manufacturer: VWR
Model: Symphony
Serial number: BE0601E0100B29C80014
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

Small freezer in hallway
Manufacturer: Revco
Model: ULT390-7ABA
Serial number: WOBB-124601-WB
DWR inventory tag number: FE102889
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

C. SAFETY PRECAUTIONS

- Use caution when moving large equipment and when handling ultra-cold items. Use freezer gloves when needed.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Paper towels or soft cloth
2. Wet/dry vacuum
3. Thermometers
4. Temperature charts
5. 70% isopropyl alcohol
6. Paper towels

E. PROCEDURES

1. Main unit:
   - Monitor temperature regularly using the thermometers inside each unit and record on log.
   - Scrape frost from doors when it builds up.
   - Check the gasket for tears or punctures.

ORIGINAL

   - Wipe gaskets with a soft cloth.
2. Condenser:
   - Clean the condenser at least every 6 months; more often if in a dusty area.
     - Open the grill.
     - Remove both filters and shake outside.
     - Vacuum the condenser.
     - Replace the filters and close the grill.
     - Monitor temperature on outside of -80° freezer and be alert to alarms.
3. Alarm battery:
   - Replace alarm battery every 12 months by a certified technician.

F. QUALITY CONTROL

- Follow appropriate maintenance to ensure proper working order of equipment[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manuals

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached temperature and maintenance log.

ORIGINAL

Large -80°C Freezer

Temperature		Defrost
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

Small Chest -80°C Freezer

Temperature		Defrost
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Autoclave Maintenance and Sterility Assurance**	
SOP #: 902	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of autoclaves, as well as the procedures of sterility assurance.

B. EQUIPMENT INFORMATION

Large autoclave in main lab
Manufacturer: Market Forge
Model: STM-EL
Serial number: 233984
DWR inventory tag number: 15443
Manufacturer contact information: mfii.com
Technical service contact number: 1-888-259-7076
Contact email: custserv@mfii.com

Small autoclave in virology lab
Manufacturer: Market Forge
Model: STM-EL
Serial number: 189741
DWR inventory tag number: N/A
Manufacturer contact information: mfii.com
Technical service contact number: 1-888-259-7076
Contact email: custserv@mfii.com

C. SAFETY PRECAUTIONS

- Use care when moving equipment.
- Autoclave will be hot after use.
- Never open autoclave when it is still pressurized.

D. EQUIPMENT AND MATERIALS REQUIRED

1. dH_2O
2. Waste water bucket
3. Autoclave sterility packets

E. PROCEDURES

1. To sterilize materials:
   - Place materials inside metal basket and slide into autoclave chamber.
   - Place waste water bucket below drain.
     - Open drain and dispose of water, close drain.
   - Fill reservoir to just below lip.
   - Close door.

ORIGINAL

        - Ensure gasket is properly sealed.
    - Choose between “fast” and “slow” settings, and set dial for 15-20 minutes.
        - Autoclave should already be set to 121°C and 20 psi.
    - Wait until cycle is completely finished before opening door.
2. Sterility assurance:
    - Remove two spore ampules from the box and label with the date the autoclave is being tested.
    - One ampule is a control. This vial will not be autoclaved and will act as a spore viability control.
    - Place the ampule in a horizontal position in the center of a load.
    - Process the load as usual.
        - When complete, open the autoclave door and allow to cool for at least 10 minutes.
    - Place both spore ampules (control and test), in the oven and assure temperature is 55°C-60°C for 48 hours.
    - Examine the indicator for any color change and record the result at 48 hours.
        - The change of color toward yellow and/or turbidity indicates bacterial growth.
        - No color change indicates adequate sterilization.
    - Sterility assurance should be monitored on an annual basis.
        - Document results on maintenance log.

F. QUALITY CONTROL

- Followed scheduled maintenance and testing schedules[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Equipment manuals

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached maintenance logs.

ORIGINAL

Large autoclave in main lab- Sterility assurance

Date Performed	Performed By	Result

ORIGINAL

Small autoclave in virology lab- Sterility assurance

Date Performed	Performed By	Result

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Scales**	
SOP #: 903	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to insure that scales are properly maintained and calibrated.

B. EQUIPMENT INFORMATION

Manufacturer: Ohaus Corporation
Model: Explorer Pro EP413D
Serial number: N1321123121797P
DWR inventory tag number: N/A
Manufacturer contact information: http://www.ohaus.com/en-US/
Technical service contact number: 1-800-672-7722
Contact email: repair@qc-services.com

C. SAFETY PRECAUTIONS

- Use care when moving and/or transporting balances.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. Paper towels

E. PROCEDURES

1. Wipe down balance with 70% isopropyl alcohol before and after each use.
2. Maintain a consistent calibration schedule[1].
   - Record calibration information on attached maintenance log.
   - Balances are suggested to be calibrated every 3 years.

F. QUALITY CONTROL

- Be sure to follow scheduled maintenance recommendations.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manual

I. REVISION HISTORY

1. Original

ORIGINAL

J. APPENDICES

- See attached maintenance log.

Scale Maintenance

Service Performed	Date Service Performed	Vendor Performing Service

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Centrifuges**	
SOP #: 904	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to perform periodic maintenance needed for centrifuges. No regularly scheduled maintenance is required.

B. EQUIPMENT INFORMATION

Small centrifuge in main lab
Manufacturer: Eppendorf
Model: 5430 R
Serial number: 5428YN606302
DWR inventory tag number: N/A
Manufacturer contact information: https://www.eppendorf.com/US-en/login/auth/on/
Technical service contact number: 1-480-244-5563
Contact email: chinchillin@eppendorf.com

Large centrifuge in main lab
Manufacturer: Brinkmann Instruments, Inc.
Model: 5810 R
Serial number: 5811 08246
DWR inventory tag number: 15090
Manufacturer contact information: https://www.eppendorf.com/US-en/login/auth/on/
Technical service contact number: 1-800-645-3050
Contact email: techserv@eppendorf.com

Centrifuge in virology
Manufacturer: Eppendorf
Model: 5410 R
Serial number: 5811-08246
DWR inventory tag number: 15123
Manufacturer contact information: https://www.eppendorf.com/US-en/login/auth/on/
Technical service contact number: 1-800-645-3050
Contact email: techserv@eppendorf.com

C. SAFETY PRECAUTIONS

- Use caution when moving or transporting centrifuges.

ORIGINAL

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. DNA Away
4. Paper towels

E. PROCEDURES

1. Wipe down centrifuge and rotors with 70% isopropyl alcohol and/or DNA Away before and after each use.
2. Perform maintenance as needed during use.
    - No scheduled maintenance is required[1].

F. QUALITY CONTROL

- Be sure to clean equipment before and after each use.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manuals

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached maintenance log.

ORIGINAL

Centrifuge Maintenance
Model: Eppendorf 5430R

Service Performed	Date Service Performed	Vendor Performing Service

ORIGINAL

Centrifuge Maintenance
Model: Brinkmann Instruments Inc 5430R

Service Performed	Date Service Performed	Vendor Performing Service

ORIGINAL

Centrifuge Maintenance
Model: 5810R

Service Performed	Date Service Performed	Vendor Performing Service

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: BioTek Epoch Plate Reader**	
SOP #: 905	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of the plate reader. A regular cleaning regimen is recommended to keep the instrument free from dust and particulates that can cause erroneous readings.

B. EQUIPMENT INFORMATION

Manufacturer: BioTek Instruments
Model: Epoch
Serial number: 240211
DWR inventory tag number: N/A
Manufacturer contact information: www.biotek.com
Technical service contact number: 888-451-5171
Contact email: TAC@biotek.com

C. SAFETY PRECAUTIONS

- Use care when moving and/or transporting plate reader.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. DNAway™
4. Paper towels

E. PROCEDURES

1. Cleaning the microplate plate carrier:
   - Turn on the Epoch plate reader and press the carrier eject button to eject the microplate carrier.
   - Turn off and unplug the reader from the power supply.
   - Moisten a clean, lint-free Kimwipe with water.
   - Wipe the plate carrier and all exposed surfaces with the moistened Kimwipe.
   - Dry plate carrier with a new, clean Kimwipe.
2. External surface disinfection:
   - Wipe external surfaces with 70% isopropyl alcohol and/or DNAway™.

F. QUALITY CONTROL

- Ensure regular cleaning of microplate plate carrier and keep dust accumulation to a minimum[1].

G. INTERPRETATION

N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Eye Wash Spray Stations**	
SOP #: 906	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to provide eyewash stations that are clean and operational in case of eye exposure to contaminants.

B. EQUIPMENT INFORMATION

Manufacturer: Bel-Art Products
Model: Science Ware
Serial number: 999320233

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Spray bottle in eye wash station
2. Fresh dH_2O

E. PROCEDURES

1. Keep plastic cover on spray bottle when not in use.
2. Inspect the spray bottle station monthly. Three separate stations exist at Fisheries Experiment Station (FES), located in the virology lab, main bacteriology lab, and in the west lab.
   - Ensure spray nozzle is not clogged by squeezing bottle and confirming steady flow of water.
   - Replace water in spray bottle with fresh dH_2O.
   - Record on attached maintenance log.

F. QUALITY CONTROL

- Stations must be inspected monthly to ensure safety and quality[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment

I. REVISION HISTORY

1. Original

ORIGINAL

J. APPENDICES

- See attached maintenance log.

Date checked & water replaced	Performed by

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Gel Electrophoresis Boxes**	
SOP #: 907	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to keep the gel electrophoresis units clean and functional.

B. EQUIPMENT INFORMATION

Manufacturer: Fisher Scientific
Model: FB-SB-1316
Serial number: 205815/238622
DWR inventory tag number: N/A
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

C. SAFETY PRECAUTIONS

- Use care when moving and/or transporting units. Unplug unit from power supply and ensure care not to spill liquid inside (Tris-borate-ethylenediaminetetraacetic acid; TBE).

D. EQUIPMENT AND MATERIALS REQUIRED

1. 1x TBE buffer
2. dH_2O
3. Paper towels
4. DNAway™
5. Fresh bleach solution

E. PROCEDURES

1. **Preparation for use**
   - Fill electrophoresis gel box with 1x TBE buffer[1].
   - Place prepared gel[2] in gel box and ensure liquid covers gel.
   - See Gel Electrophoresis and Visualization[2] for further use.
2. **Thoroughly clean and disinfect gel box after each use.**
   - Remove lid of gel box (electrodes will remain attached to lid) and leave in gel hood.
   - Dispose used TBE buffer down sink.
   - Dry gel box using paper towels.
   - Thoroughly wipe inside and outside of gel box (including lid) with DNAway™ and/or fresh bleach solution. Box can sit in bleach bath if desired.
   - Rinse with dH_2O.
   - Let air dry or dry thoroughly with paper towel.

ORIGINAL

F. QUALITY CONTROL

- Be sure to disinfect and clean box between each use[3].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 832, 1X Tris Borate EDTA (TBE) Buffer
2. SOP# 718, Gel Electrophoresis and Visualization
3. SOP# 202, General Use and Maintenance of Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Gel Box Power Supply**	
SOP #: 908	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to supply current to run gel electrophoresis and for light routine maintenance of power box.

B. EQUIPMENT INFORMATION

Manufacturer: BioRad
Model: PwerPak HC
Serial number: 043BR 17840
DWR inventory tag number: N/A
Manufacturer contact information: www.bio-rad.com
Technical service contact number: 1-800-424-6723
Contact email: support@bio-rad.com

C. SAFETY PRECAUTIONS

- Make sure unit is unplugged from power source before performing maintenance.

D. EQUIPMENT AND MATERIALS REQUIRED

1. DNAway™
2. Fresh bleach solution
3. Paper towels

E. PROCEDURES

1. To use:
   - Turn power box on (side switch)
   - Adjust voltage as necessary (use arrows to navigate and adjust).
   - Set time as necessary (use arrows to navigate and adjust).
   - Push “run” button to start power supply.
   - Confirm successful power supply by checking formation of bubbles within TBE buffer in gel box.
2. After running, push “stop” button and turn off power box (side switch).
3. Wipe down surface of power supply with DNAway™ and/or bleach solution after each use.
4. Keep a 3” clearance behind the power supply; do not block the vented area.

F. QUALITY CONTROL

- Keep power supply box clear and clean[1].

G. INTERPRETATION

N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Fume Hoods And Biosafety Cabinets**	
SOP #: 909	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to ensure the fume hoods and biosafety cabinets are properly maintained for efficient operation.

B. EQUIPMENT INFORMATION

Fume hood in histology lab
Manufacturer: Labonco
Model: Labonco Fume Hood
Serial number: Not available
DWR inventory tag number: 15057
Manufacturer contact information: www.labonco.com
Technical service contact number: 1-800-821-5525
Contact email: Contact form www.labonco.com

Biosafety cabinet/laminar flow hood in virology lab
Manufacturer: Nuaire
Model: NU-425-600
Serial number: 16880TT
DWR inventory tag number: 14004
Manufacturer contact information: www.nuaire.com
Technical service contact number: 763-553-1270
Contact email: Contact form at www.nuaire.com

Bench top biosafety cabinet/laminar flow hood in virology lab (used for PCR)
Manufacturer: Airclean
Model: Airclean 600 PCR Workstation
Serial number: AC632FLUVC-42675
DWR inventory tag number: N/A
Manufacturer contact information: https://www.aircleansystems.com/
Technical service contact number: 1- 800-849-0472
Contact email: service@aircleansystems.com

Bench top biosafety cabinet in histology lab (used for gel loading)
Manufacturer: Airclean
Model: Airclean 600 PCR Workstation
Serial number: AC632DB-3965
DWR inventory tag number: N/A
Manufacturer contact information: https://www.aircleansystems.com/
Technical service contact number: 1- 800-849-0472
Contact email: service@aircleansystems.com

ORIGINAL

Bench top biosafety cabinet in necropsy lab (used for PCR)
Manufacturer: Airclean
Model: Airclean 600 PCR Workstation
Serial number: AC632DB-3966
DWR inventory tag number: N/A
Manufacturer contact information: https://www.aircleansystems.com/
Technical service contact number: 1- 800-849-0472
Contact email: service@aircleansystems.com

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. HEPA filters (for use in bench top biosafety cabinet/laminar flow hood in virology)
2. UV light bulbs (for use in all biosafety cabinets)
3. DNAway™
4. Fresh bleach solution
5. 70% isopropyl alcohol
6. Paper towels

E. PROCEDURES

1. Fume hood in histology lab:
   - Ensure air flow is on before use.
   - Maintain fluorescent light source as needed.
   - Requires certification of proper function by outside vendor (see attached maintenance log and certification records) annually[1].
2. Biosafety cabinet/laminar flow hood in virology lab:
   - Wipe down all surfaces with 70% isopropyl alcohol and/or DNAway™ or bleach if needed after each use.
   - Turn UV light on after each use for at least 20 minutes.
   - Requires certification of proper function by outside vendor (see attached maintenance log and certification records) annually.
3. Bench top biosafety cabinet/laminar flow hood in virology lab:
   - Wipe down all surfaces with 70% isopropyl alcohol and/or DNAway™ or bleach if needed after each use.
   - Turn UV light on after each use for at least 20 minutes.
   - Replace HEPA filter on an annual basis or as designated by the unit (see attached maintenance log).
   - Replace UV light as necessary (see attached maintenance log).
4. Bench top biosafety cabinets in histology and necropsy labs:
   - Wipe down all surfaces with 70% isopropyl alcohol and/or DNAway™ or bleach if needed after each use.
   - Turn UV light on after each use for at least 20 minutes.
   - Replace UV light as necessary (see attached maintenance log).

F. QUALITY CONTROL

- Be sure to follow scheduled maintenance recommendations.

ORIGINAL

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manuals

I. REVISION HISTORY

- Original

J. APPENDICES

- See attached maintenance logs.

Fume Hood (In Histology) Maintenance-- Certification

Date Service Performed	Vendor Performing Service

ORIGINAL

Biosafety Cabinet/Laminar Flow Hood (In Virology) Maintenance-- Certification

Date Service Performed	Vendor Performing Service	UV Light Maintenance

ORIGINAL

Bench Top Biosafety Cabinet/Laminar Flow Hood (In Virology) Maintenance

HEPA Filter Replacement Date	Performed By	UV Light Maintenance	Performed By

ORIGINAL

Biosafety Cabinet (In Histology) Maintenance

Date UV Bulb Replaced	Performed By

ORIGINAL

Biosafety Cabinet (In Necropsy) Maintenance

Date UV Bulb Replaced	Performed By

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Hot Plates, Microwave and Stirrers**	
SOP #: 910	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to keep the stir/hot plates clean and functional.

B. EQUIPMENT INFORMATION

Stir/hot plate in histology
Manufacturer: VWR
Model: 730ADV
Serial number: 090520002
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

Slide warmer in histology
Manufacturer: Electrothermal
Model: Electrothermal Slide drying bench
Serial number: 10067897
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

Microwave in histology
Manufacturer: Magic Chef
Model: MCM1HOB
Serial number: 15659200391
DWR inventory tag number: N/A
Manufacturer contact information: https://mcappliance.com/magic-chef
Technical service contact number: 1-888-775-0202

C. SAFETY PRECAUTIONS

- Use care when cleaning hot plates, surfaces may be hot!
- Unplug equipment before cleaning and moving.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Paper towels
2. Nylon scrubbing pad, if necessary
3. 70% isopropyl alcohol

ORIGINAL

E. PROCEDURES

1. Clean spills from the surface as soon as possible.
    - If surface is hot, wait until cool to touch.
2. When necessary, clean with a nylon scrubbing pad.
3. Wipe down after each use with 70% isopropyl alcohol.

F. QUALITY CONTROL

- Be sure to keep all equipment clear and clean[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Incubators**	
SOP #: 911	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to ensure the incubators are cleaned and defrosted and kept at the proper temperatures. Ice builds up behind the lining of the incubators and can affect the coils.

B. EQUIPMENT INFORMATION

Bench top low temperature incubator in main lab
Manufacturer: EchoTherm
Model: IN30
Serial number: 518002
DWR inventory tag number: 10983
Manufacturer contact information: TorreyPinesScientific.com
Technical service contact number: 866-573-9104
Contact email: techsupport@torreypinesscientific.com

Large low temperature incubator in main lab
Manufacturer: Precision
Model: Low Temp 815
Serial number: 35AX-3
DWR inventory tag number: FE102890
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

Bench top high temperature (oven) in histology lab
Manufacturer: Labline
Model: 120
Serial number: 1091-0061
DWR inventory tag number: N/A
Manufacturer contact information: https://www.lablineinstruments.html
Technical service contact number: 0484-2776582

Large low temperature incubator in virology lab #2
Manufacturer: VWR
Model: BOD Low Temp
Serial number: 04033710
DWR inventory tag number: 25496R
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

ORIGINAL

Large low temperature incubator in virology lab #1
Manufacturer: Fisher Scientific
Model: Low Temp Incubator
Serial number: 311N0269
DWR inventory tag number: FE102898
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

Small high temperature incubator in virology lab
Manufacturer: Fisher Scientific
Model: Low Temp Incubator
Serial number: 311040-11
DWR inventory tag number: FE102895
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

C. SAFETY PRECAUTIONS

- Unplug equipment before moving and for major servicing.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 70% isopropyl alcohol
2. Fresh bleach solution
3. Paper towels
4. Cloth towels
5. Pan/towels to catch water during defrosting
6. Wet dry vacuum if necessary
7. Calibrated thermometer

E. PROCEDURES

1. Defrosting incubators:
   - As needed, but at least on an annual basis.
   - Remove the contents of the incubator.
   - Unplug incubator and leave door open slightly.
   - Place towels or pan underneath and around incubator to catch water as it defrosts.
   - Leave incubator unplugged until sufficiently defrosted.
   - Plug back in, return to original temperature, and replace contents.
   - Clean up melted water.
   - Record on maintenance log.
2. Cleaning/disinfecting incubators:
   - As needed, but at least on an annual basis.
   - Remove the contents of the incubator.
   - Wipe down all surfaces with 70% isopropyl alcohol and/or fresh bleach solution.
   - Record on maintenance log.

ORIGINAL

3. Monitoring temperature:
   - Should be performed regularly.
   - Use a calibrated thermometer occasionally to check the temperature.
   - Record on maintenance log.

F. QUALITY CONTROL

- Be sure to follow scheduled maintenance and defrost/disinfect as necessary[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual for each unit

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached maintenance logs.

Bench top low temperature incubator in main lab

Defrost		**Disinfect**		**Temperature check**	
Date	Performed by	Date	Performed by	Date	Performed by

ORIGINAL

Large low temperature incubator in main lab

Defrost		Disinfect		Temperature check	
Date	Performed by	Date	Performed by	Date	Performed by

ORIGINAL

Bench top high temperature (oven) in histology lab

Defrost		Disinfect		Temperature check	
Date	Performed by	Date	Performed by	Date	Performed by

ORIGINAL

Large low temperature incubator in virology lab #2

Defrost		Disinfect		Temperature check	
Date	Performed by	Date	Performed by	Date	Performed by

ORIGINAL

Large low temperature incubator in virology lab #1

Defrost		Disinfect		Temperature check	
Date	Performed by	Date	Performed by	Date	Performed by

ORIGINAL

Small high temperature incubator in virology lab

Defrost		**Disinfect**		**Temperature check**	
Date	Performed by	Date	Performed by	Date	Performed by

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Microscopes**	
SOP #: 912	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of the microscopes.

B. EQUIPMENT INFORMATION

Main fluorescent microscope

Manufacturer: Olympus
Model: 8X40F4
Serial number: 8K03341
DWR inventory tag number: 14996
Manufacturer contact information: http://www.olympus-lifescience.com
Technical service contact number: 1-800-225-8330
Contact email: Kathleen.karmel@olympus-ossa.com

Main light microscope (with fluorescent filter)

Manufacturer: Olympus
Model: BX51
Serial number: 1A12425
DWR inventory tag number: N/A
Manufacturer contact information: http://www.olympus-lifescience.com
Technical service contact number: 1-800-225-8330
Contact email: Kathleen.karmel@olympus-ossa.com

Double headed microscope in necropsy

Manufacturer: Reichert
Model: Microstar IV
Serial number: BE311933
DWR inventory tag number: 13891
Manufacturer contact information: http://www.reichert.com/
Technical service contact number: 1-888-849-8955
Contact email: Online form

Traveling light microscope

Manufacturer: Zeiss
Model: N/A
Serial number: 470801-9097
DWR inventory tag number: 14846
Manufacturer contact information: www.zeiss.com/microscopy
Technical service contact number: 1-800-233-2343
Contact email: info.microscopy.us@zeiss.com

ORIGINAL

Dissecting microscope in necropsy
Manufacturer: Vistavision
Model: N/A
Serial number: 0908234
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

Inverted light microscope in virology
Manufacturer: Fisher Scientific
Model: MicroMaster
Serial number: FW9716-1007
DWR inventory tag number: 25495
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

Inverted light microscope in virology clean room
Manufacturer: American Optical
Model: 1810
Serial number: 1525
DWR inventory tag number: 10090
Manufacturer contact information: No longer intact. See Marty's Microscope Service
Technical service contact number: 801-582-6144
Contact email: N/A

C. SAFETY PRECAUTIONS

- N/A

D. EQUIPMENT AND MATERIALS REQUIRED

1. Bulbs
2. Lens cleaning solution
3. Lint free wipes (Kimwipes)
4. Microscope covers

E. PROCEDURES

1. Clean oil from objectives after each use.
   - Remove excess oil with a Kimwipe.
   - Clean the objective with lens cleaner and Kimwipe.
   - Clean the recesses of the objective lens with a cotton swab and lens cleaner as needed.
   - Wipe the surface of the objective lens with a dry Kimwipe
   - Clean oil off of the platform with lens cleaner and a Kimwipe.
   - Clean the eyepieces with Kimwipe and lens cleaner.
2. Cover microscopes after each use and make sure all equipment is turned off.

ORIGINAL

3. If necessary, user or microbiologist may make minor adjustments and bulb changes.
    - Bulbs and fluorescent filters may require the service of a microscope technician.
4. Periodically schedule a professional service to clean and adjust all microscopes, see attached microscope maintenance log.

F. QUALITY CONTROL

- Ensure proper maintenance of all microscopes[1]. Scopes are calibrated and serviced annually by Marty's Microscope Service: 5199 E. Silver Oak Road Salt Lake City, UT 84108.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manuals

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached maintenance log.

ORIGINAL

Microscope Maintenance

Microscope	Service Performed	Date Service Performed	Vendor Performing Service

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: pH Probes**	
SOP #: 913	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods required to properly maintain the pH probe to achieve accurate measurement of pH.

B. EQUIPMENT INFORMATION

Manufacturer: ThermoFisher
Model: Orion Star AIII
Serial number: J4424
DWR inventory tag number: N/A
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

C. SAFETY PRECAUTIONS

- Use care when moving and/or transporting pH probe and digital display.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Buffers (pH 4, pH 7, and pH 10)
2. Distilled water (dH_2O)
3. Probe storage buffer

E. PROCEDURES

1. To calibrate pH probe:
   - Should be done each time before use.
   - Follow instructions in manual and on digital display to calibrate.
   - Use fresh buffer solutions each time and rinse with dH_2O between buffers.
2. To take a reading:
   - Gently remove storage cap and rinse with dH_2O.
   - Gently place probe in solution to be tested.
   - Follow instructions on digital display to take a reading.
3. Thoroughly rinse with dH_2O after each use and gently replace storage cap.
   - Ensure there is adequate storage buffer in storage container.
4. Add potassium chloride (KCl) into the probe junction as necessary.

F. QUALITY CONTROL

- Be sure to calibrate probe before each use and keep probe clean and clear[1].

G. INTERPRETATION

N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Pipettors**	
SOP #: 914	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of pipettors.

B. EQUIPMENT INFORMATION

Main lab pipettors

Manufacturer: Eppendorf
Model: Multiple
Serial number: Multiple
DWR inventory tag number: N/A
Manufacturer contact information: https://www.eppendorf.com/US-en/login/auth/on/
Technical service contact number: 1-800-645-3050
Contact email: techserv@eppendorf.com

Molecular work pipettors

Manufacturer: VWR (Gel Pipettes)
Model: Multiple
Serial number: Multiple
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

Virology pipettors

Manufacturer: Drummond
Model: Multiple
Serial number: Multiple
DWR inventory tag number: N/A
Manufacturer contact information: drummondsci.com
Technical service contact number: 1-800-523-7480
Contact email: info@drummondsci.com

C. SAFETY PRECAUTIONS

- Take care when handling pipettors, do not drop.

D. EQUIPMENT AND MATERIALS REQUIRED

1. 70% isopropyl alcohol
2. Fresh bleach solution
3. DNAway™
4. Paper towels

ORIGINAL

5. 0.2 µm hydrophobic membrane filter

E. PROCEDURES

1. Keep all pipettors clean and disinfected.
    - When working with DNA or RNA use bleach and/or DNAway™ followed by 70% isopropyl alcohol.
    - Wipe surfaces with 70% isopropyl alcohol after each use.
2. Keep electronic pipettes charged.
3. Use barrier tips for molecular work.
4. Pipettors must be calibrated at least every three years[1], on an annual basis is preferred.
5. When using serological pipette aids and filter becomes wet, unscrew filter casing and replace wet filter with new 0.2 µm hydrophobic membrane filter.

F. QUALITY CONTROL

- Ensure proper calibration schedule and maintenance[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manuals

I. REVISION HISTORY

1. Original

J. APPENDICES

- See attached calibration records.

ORIGINAL

Pipettors

Service Performed	Date Service Performed	Vendor Performing Service

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Platform Rockers**	
SOP #: 915	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the procedures required to care for and maintain platform rockers.

B. EQUIPMENT INFORMATION

Manufacturer: Stoval
Model: Belly Button
Serial number: BBUV012568
DWR inventory tag number: N/A
Manufacturer contact information: www.denvillescientific.com
Technical service contact number: 1-800-453-0385
Contact email: support@hbiosci.com

C. SAFETY PRECAUTIONS

- Use care when moving and/or transporting shakers.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. Paper towels

E. PROCEDURES

1. Keep the platform clean, and turned off when not in use.
    - Wipe surfaces of rocker with 70% isopropyl alcohol as necessary.
2. Remove platform rocker from incubator when defrosting.

F. QUALITY CONTROL

- Keep equipment clean and clear[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual

I. REVISION HISTORY

1. Original

J. APPENDICES

ORIGINAL

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Refrigerators and Freezers**	
SOP #: 916	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of laboratory refrigerators and freezers.

B. EQUIPMENT INFORMATION

White sample fridge/freezer combo in main lab
Manufacturer: Haier
Model: PRT521SACW
Serial number: BL007500U00TAA5Q0705
DWR inventory tag number: N/A
Manufacturer contact information: www.haieramerica.com
Technical service contact number: 1-877-337-3639
Contact email: customercare@haieramerica.com

Black reagent fridge/freezer combo in main lab #1
Manufacturer: Haier
Model: RRCS25SDAB
Serial number: 14208072352603000179
DWR inventory tag number: 19943
Manufacturer contact information: www.haieramerica.com
Technical service contact number: 1-877-337-3639
Contact email: customercare@haieramerica.com

Black reagent fridge/freezer combo in main lab #2
Manufacturer: Haier
Model: RRCS25SDAB
Serial number: 14208072352603000193
DWR inventory tag number: 19942
Manufacturer contact information: www.haieramerica.com
Technical service contact number: 1-877-337-3639
Contact email: customercare@haieramerica.com

White upright freezer in break room
Manufacturer: WC. Wood
Model: F425W
Serial number: 40170886CQ
DWR inventory tag number: FE102929
Manufacturer contact information: www.searspartsdirect.com
Technical service contact number: 1-800-491-3457
Contact email: Online form

ORIGINAL

White fridge/freezer combo in virology
Manufacturer: Amana
Model: TX178VC
Serial number: 0010258426
DWR inventory tag number: FE102895
Manufacturer contact information: www.amana.com
Technical service contact number: 1-866-587-2002
Contact email: Online form

White sample fridge combo in virology
Manufacturer: Crosley
Model: WCR18/W3
Serial number: 40111494HP
DWR inventory tag number: 19872
Manufacturer contact information: www.crosley.com
Technical service contact number: 1-704-956-2523
Contact email: Online form

PCR positive control freezer
Manufacturer: Whirlpool
Model: EL02PPXMQ
Serial number: EER1636173
DWR inventory tag number: FE102892
Manufacturer contact information: www.whirlpool.com
Technical service contact number: 1-866-698-2538
Contact email: Online form

Histology freezer
Manufacturer: WC. Wood
Model: MU07W
Serial number: 60965109MQ
DWR inventory tag number: FE102893
Manufacturer contact information: www.searspartsdirect.com
Technical service contact number: 1-800-491-3457
Contact email: Online form

PCR sample fridge combo in histology
Manufacturer: Kenmore
Model: 5648951720
Serial number: 51200967
DWR inventory tag number: FE102893
Manufacturer contact information: www.kenmore.com
Technical service contact number: 1-888-536-4505
Contact email: Online form

C. SAFETY PRECAUTIONS

- Use care when moving large equipment.

ORIGINAL

D. EQUIPMENT AND MATERIALS REQUIRED

1. Pan to collect water when defrosting
2. 70% isopropyl alcohol
3. Paper towels
4. Wet/dry vacuum
5. Thermometers
6. Temperature logs

E. PROCEDURES

1. Monitor temperatures regularly with thermometers inside each unit.
2. Defrost when frost is developing a layer in the freezer.
3. Wipe surfaces with 70% isopropyl alcohol when defrosted.

F. QUALITY CONTROL

- Ensure proper temperature regularly[1]. For general onsite repairs, contact the service depot at 435-753-4243.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manuals

I. REVISION HISTORY

1. Original

APPENDICES

- See attached maintenance and temperature logs.

ORIGINAL

White sample fridge/freezer combo in main lab

Temperature		**Defrost**
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

Black reagent fridge/freezer combo in main lab #1

Temperature		**Defrost**
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

Black reagent fridge/freezer combo in main lab #2

Temperature		**Defrost**
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

White upright freezer in break room

Temperature		**Defrost**
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

White fridge/freezer combo in virology

Temperature		Defrost
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

White sample fridge combo in virology

Temperature		**Defrost**
Temperature	Date and Performed by	Date and Performed by

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Sonicator**	
SOP #: 917	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of the sonicator.

B. EQUIPMENT INFORMATION

Manufacturer: VWR
Model: Branson Sonifier 450
Serial number: BI70070Y
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

C. SAFETY PRECAUTIONS

- Use care when handling the sonicator so as not to injure oneself.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. Fresh bleach solution
4. Paper towels

E. PROCEDURES

1. Thoroughly clean and disinfect sonicator before and after each use.
   - Wipe all surfaces with fresh bleach solution followed by 70% isopropyl alcohol.

F. QUALITY CONTROL

- Make sure equipment is disinfected before and after each use[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manual

I. REVISION HISTORY

1. Original

J. APPENDICES

ORIGINAL

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title**: Stomacher**	
SOP #: 918	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of the stomacher.

B. EQUIPMENT INFORMATION

Manufacturer: Seward
Model: 80Mark 2
Serial number: 40806
DWR inventory tag number: 19687R
Manufacturer contact information: seward.co.uk
Technical service contact number: 1-631-337-1808
Contact email: info@sewardusa.com

C. SAFETY PRECAUTIONS

- Use care when moving and/or transporting stomacher.
- Use caution when operating stomacher so as not to injure oneself.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. Paper towels

E. PROCEDURES

1. Wipe down all surfaces with 70% isopropyl alcohol after each use.

F. QUALITY CONTROL

- Keep equipment clean and clear[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah

Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Thermocyclers**	
SOP #: 919	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of thermocyclers.

B. EQUIPMENT INFORMATION

BioRad Thermocycler in main lab
Manufacturer: Bio Rad
Model: MyCycler
Serial number: 580BR08240
DWR inventory tag number: N/A
Manufacturer contact information: www.bio-rad.com
Technical service contact number: 1-800-424-6723
Contact email: support@bio-rad.com

BioRad Thermocycler in virology lab
Manufacturer: BioRad
Model: MyCycler
Serial number: 580BR4439
DWR inventory tag number: N/A
Manufacturer contact information: www.bio-rad.com
Technical service contact number: 1-800-424-6723
Contact email: support@bio-rad.com

Eppendorf Thermocycler in virology lab
Manufacturer: Eppendorf
Model: MasterCycler
Serial number: 11610
DWR inventory tag number: N/A
Manufacturer contact information: https://www.eppendorf.com/US-en/login/auth/on/
Technical service contact number: 1-800-645-3050
Contact email: techserv@eppendorf.com

Real-Time Thermocycler in histology lab
Manufacturer: Applied Biosystems
Model: StepOne Plus
Serial number: 2720010666
DWR inventory tag number: N/A
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

ORIGINAL

C. SAFETY PRECAUTIONS

- Use care when moving thermocyclers.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. DNAway™
4. Paper towels

E. PROCEDURES

1. Wipe down external surfaces with DNAway™ followed by 70% isopropyl alcohol after each use.
2. Calibrate each thermocycler as deemed necessary[1].
    - Follow suggestions in user's manual and contact manufacturer's support for assistance.

F. QUALITY CONTROL

- Keep thermocyclers clean and disinfected.

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. User manuals

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Vortexes**	
SOP #: 920	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of vortexes.

B. EQUIPMENT INFORMATION

Manufacturer: VWR
Model: Digital vortex, Vortex 2
Serial number: Assorted
DWR inventory tag number: N/A
Manufacturer contact information: us.vwr.com
Technical service contact number: 1-888-897-5463
Contact email: technicalproductSupportNA@vwr.com

Manufacturer: Fisher Scientific
Model: MiniRoto S56
Serial number: Assorted
DWR inventory tag number: N/A
Manufacturer contact information: https://www.fishersci.com/us/en/home.html
Technical service contact number: 1-800-955-6288
Contact email: Heather.Loughton@thermofisher.com

C. SAFETY PRECAUTIONS

- Use caution when moving and/or transporting vortexes.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. Paper towels

E. PROCEDURES

1. Ensure unit is turned off and/or unplugged after use.
2. Wipe surfaces with 70% isopropyl alcohol after each use.

F. QUALITY CONTROL

- Ensure equipment is kept clean and clear[1].

G. INTERPRETATION

N/A

ORIGINAL

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual

I. REVISION HISTORY

1. Original

J. APPENDICES

- **N/A**

ORIGINAL

State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan UT 84321

Title: **Water Baths**	
SOP #: 921	Date Initiated: 3/30/2017
Approved by: Cristi Swan	Date Last Revised:
Author: Danielle Van Vliet & Maria Hansen	Date Last Reviewed: 3/30/2017
Laboratory Section: Instrumentation	Date Next Review Due: 3/30/2020

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to describe the methods of proper care and maintenance of the water bath.

B. DEQUIPMENT INFORMATION

Large water bath/shaker in main lab #1
Manufacturer: Precision
Model: Shaker Bath Model 50
Serial number: 801081454
DWR inventory tag number: 19438
Manufacturer contact information: LabCare America
Technical service contact number: 1-800-519-7971
Contact email: gv-contact@microprecision.com

Large water bath/shaker in main lab #2
Manufacturer: Precision
Model: Shaker Bath Model 50
Serial number: 697010106
DWR inventory tag number: N/A
Manufacturer contact information: LabCare America
Technical service contact number: 1-800-519-7971
Contact email: gv-contact@microprecision.com

C. SAFETY PRECAUTIONS

- Use care when moving water baths.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Manufacturer's equipment manual
2. 70% isopropyl alcohol
3. Bleach
4. dH_2O
5. Waste water bucket
6. Wrench
7. Paper towels

E. PROCEDURES

1. Empty water bath after each use.
   - Place waste water bucket below drain.
   - Use wrench to loosen drain stopper and let water bath drain.

ORIGINAL

2. Disinfect water bath after each use.
    - Squirt bleach into water bath.
    - Thoroughly rinse with fresh dH_2O.

F. QUALITY CONTROL

- Ensure proper disinfection after each use[1].

G. INTERPRETATION

N/A

H. ASSOCIATED DOCUMENTS/REFERENCES

1. SOP# 202, General Use and Maintenance of Equipment
2. Manufacturer's manual

I. REVISION HISTORY

1. Original

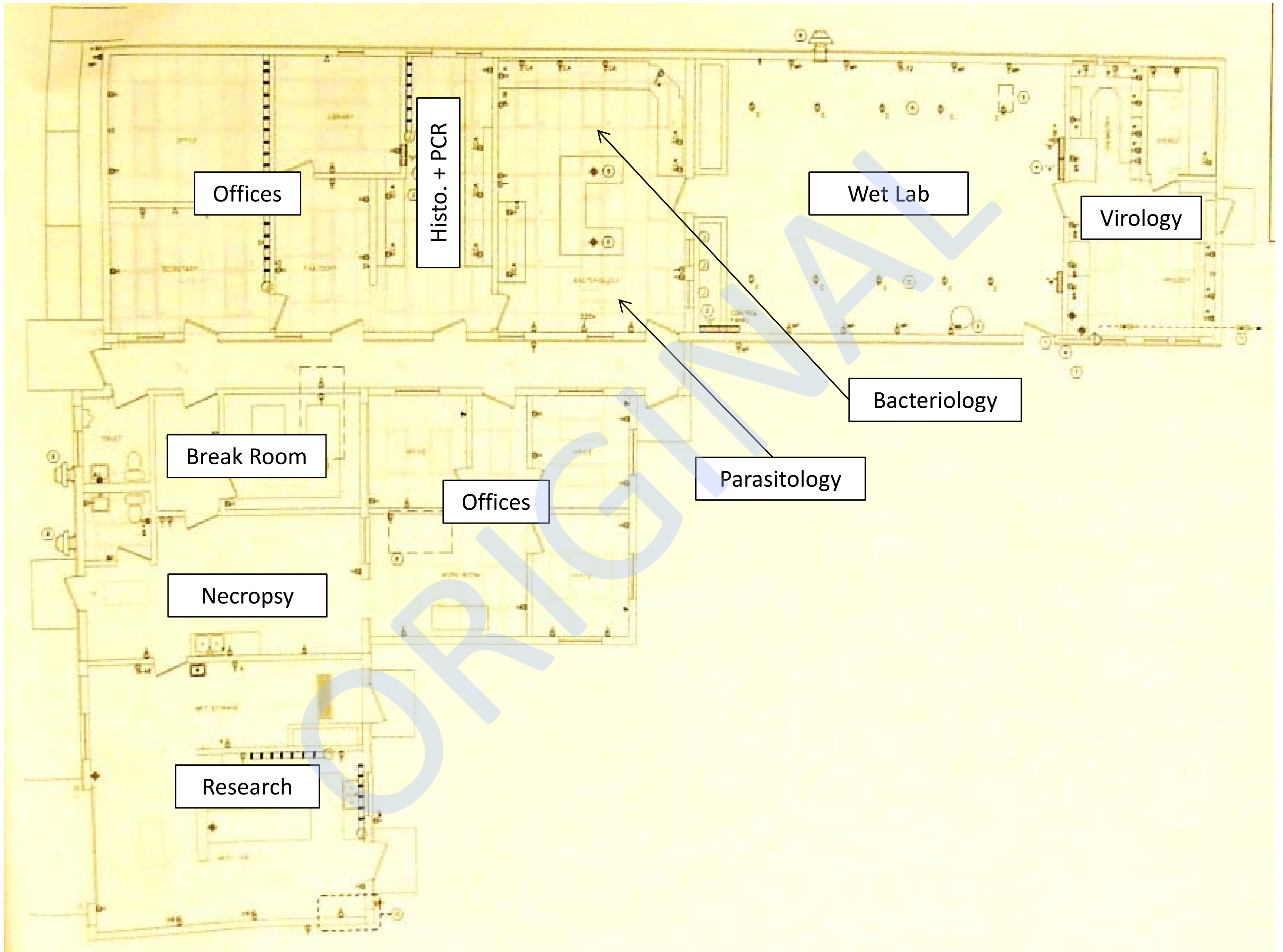
J. APPENDICES

- N/A

ORIGINAL

FES Blueprint

ORIGINAL



Offices
Histo. + PCR
Wet Lab
Virology
Bacteriology
Parasitology
Break Room
Offices
Necropsy
Research
ORIGINAL

Personnel CV

ORIGINAL

Curriculum Vitae

WADE P. CAVENDER

3673 South 100 West
Nibley, Utah 84321

Email: wadecavender@utah.gov

Office: 435-752-1066
Cell: 435-720-2784

Education:

2003 Master of Science/Fish Health and Aquaculture, Department of Natural Resources, University of Idaho, Moscow, Idaho

1999 Bachelor of Science/Fisheries Management and Aquaculture, Department of Natural Resources, University of Idaho, Moscow, Idaho

1994 Associate of Science, Fish and Wildlife Management, Department of Natural Resources, Hocking College, Nelsonville, Ohio

Professional Affiliations and Certifications:

Certified Fish Pathologist (Fish Health Section/American Fisheries Society)
Certified Aquatic Animal Health Inspector (Fish Health Section/American Fisheries Society)
Executive Secretary for Professional Standards Committee (Fish Health Section/American Fisheries Society)
Fish Health Section/American Fisheries Society member
Utah Chapter of the American Fisheries Society member

Employment History:

Utah Division of Wildlife (UDWR) 1/2016-Present
Fisheries Experiment Station (FES), Logan, UT 84321

FES Supervisor/Fish Pathologist
Currently serving as program supervisor for Utah's Fisheries Experiment Station (FES). Responsibilities include coordinating the technical research, fish health and hatchery components of FES, with emphasis on infectious disease prevention and monitoring. Supervise 12 UDWR employees. Serving as a member of the Aquatic Section administrative staff and Fish Health Policy Board. Assist and advise in the development of policies and procedures relative to fish culture and fish health. Provide recommendations in matters related to fish health including live fish transfers, aquatic animal importation, disease management of captive and wild stocks of fish and best management practices in fish culture operations. Provide diagnostic services to state culture facilities, wild fisheries, and institutional aquaculture. Serve as the Investigational New Animal Drug (INAD) monitor and provide therapeutic treatment recommendations in accordance with state and federal guidelines for safe and effective legal use of approved compounds. Prepare and provide presentations during Fish Health Policy Board, Division of Wildlife and other professional

ORIGINAL

meetings. Evaluate data, prepare reports, research and funding proposals, maintain annual budgets, perform annual employee evaluations and correspond with state and federal agencies regarding research, aquaculture and fish health issues.

Utah Division of Wildlife 3/2005-12/2015
Fisheries Experiment Station (FES), Logan, UT 84321

Fish Health Specialist-Aquatic Research/Pathology Program Coordinator
Program supervisor and laboratory manager for Utah's fish pathology/diagnostic laboratory. Duties include providing a comprehensive program of fish health inspection and certification services for State fish hatcheries and free ranging fish populations. Schedule inspections and oversee the collection and transport of samples to Utah's Fish Health Center for processing in accordance with legal requirements and Blue Book Standards as defined by the Fish Health Section/American Fisheries Society. Supervise a team of 3 microbiologists and 1 technician during the processing of samples using laboratory methods that include necropsies, cell culture, biochemical analysis, polymerase chain reaction analysis (PCR), histopathology, antibiotic sensitivity testing and fluorescent antibody testing to identify viral, bacterial and parasitic fish pathogens. Provide diagnostic and troubleshooting services for State fish hatcheries and free ranging fish populations. Serve as the Investigational New Animal Drug (INAD) monitor and provide therapeutic treatment recommendations in accordance with state and federal guidelines for safe and effective legal use of approved compounds. Provide research capabilities to address fish health issues such as vaccine development, improved treatment methods and best management practices with an emphasis on infections disease monitoring and prevention. Supervise the collection of samples for Utah's Whirling Disease survey and participate in specimen examination using PCR analysis to monitor parasite distribution. Assist and advise in the development of policies and procedures relative to fish culture, fish health and permitting for the movement of live fish and gametes within and across State borders. Provide training for hatchery personnel on disease prevention and best management practices. Attend and give presentations during professional meetings. Prepare and present annual summary and frequent updates to Utah's Fish Health Policy Board and Division of Wildlife aquatic staff. Prepare reports, research and funding proposals, maintain annual budgets, perform annual employee evaluations and correspond with state and federal agencies regarding aquaculture and fish health issues.

ORIGINAL

Arizona Game and Fish Department 2/2004-2/2005
Pinetop Fish Health Center, Pinetop, AZ 85935

Fish Health Specialist/Program Coordinator
Program supervisor and laboratory manager for Arizona's fish health program and fish pathology/diagnostic laboratory. Responsible for providing a comprehensive program of fish health inspection and certification services for State fish hatcheries and free ranging fish populations. Scheduling inspections, collection and transport of samples to Arizona's Fish Health Center for processing in accordance with legal requirements and Blue Book Standards as defined by the Fish Health Section/American Fisheries Society. Served as primary laboratory personnel responsible for performing general necropsies, PCR, cell

culture, biochemical assays and confirmatory tests to identify viral, bacterial and parasitic fish pathogens. Provided diagnostic and troubleshooting services for State fish hatcheries, free ranging fish populations and research staff by diagnosing and recommending treatment/management solutions for statewide fish health issues. Served as monitor for the legal use of Chloramine-T through the Investigational New Animal Drug (INAD) program, which is administered by the US Fish and Wildlife Service's Aquatic Animal Drug Approval Program. Assist and advise in the development of policies and procedures related to aquaculture, fish health and permitting for the movement of live fish and gametes within and across State borders. Part-time training officer for hatchery personnel on disease prevention and best management practices. Draft reports, maintain annual budgets and correspond with state, federal and private agencies regarding aquaculture and fish health issues.

University of Idaho 1/2001-10/2003
Moscow, ID 83844-1136

Graduate Student
Coordinate sentinel rainbow trout exposures within the Salmon, Snake and Clearwater rivers of Idaho to identify the distribution *Myxobolus cerebralis* within these drainages. Determine infection prevalence using histology, pepsin trypsin digest and PCR analysis. Developed a real-time Quantitative Polymerase Chain Reaction (QPCR) method to determine presence and infection intensity in rainbow trout. Designed PCR primers and probes based on the 18s and Hsp 70 sequences, optimized QPCR conditions, analyze results, interpret data in relation to current diagnostic methods and published results in peer reviewed literature. Also served as a teaching assistant during laboratory and lecture portion of undergraduate courses including aquaculture and fish health management. Formulated study plans and guided students through the process of rearing rainbow trout and formulating feed rations to reach outlined production plans in an aquaculture facility. Students were also guided through steps to identify potential fish health problems and solutions including necropsy procedures, monitoring water quality, basic bacteriology, virology, parasitology and treatment options.

Pacific State Marine Fisheries Commission on contract to the 4/2000-1/2001
National Marine Fisheries Service
Pasco, WA 99301

Fish Biologist
Organize, operate and maintain radio telemetry project for Pacific lamprey on the Columbia River. Field duties included trapping, anesthetizing, and surgically implanting adult lamprey with radio transmitters. Test fish were monitored (boat and truck) with SRX 400 receivers to examine passage efficiency through the lower Columbia River hydropower system. Additional fish were pit tagged and released into a 7000-gallon velocity flume to examine swimming endurance and passage efficiency in a laboratory setting. Draft reports and other written material as needed.

Washington State University 8/1999-5/2000
Pullman, WA 99164-6310

ORIGINAL

Teaching Assistant/Part-Time Student
Administer laboratory portion of horse production and livestock feeds and feeding to undergraduate students. Duties also included lecturing and grading exams as needed.

University of Idaho 5/1999-8/1999
Moscow, ID 83844-1136

Fisheries Technician
Trapping, anesthetizing and pit tagging Pacific lamprey to examine passage efficiency through the Columbia River hydropower system. Tests were performed by manipulating flow and structure configurations in a 7000-gallon velocity flume.

University of Idaho 8/1997-5/1999
Moscow, ID 83844-1136

Laboratory Manager
Manage daily operations in a fish health laboratory including ordering supplies, maintaining equipment and working with current graduate student. Primary laboratory objectives included isolating and identifying bacterial fish pathogens using biochemical testing methods.

University of Idaho 5/1997-8/1997
Moscow, ID 83844-1136

Fisheries Technician
Trapping, anesthetizing and surgically implanting radio transmitters in Pacific Lamprey. Trapping, anesthetizing and tagging adult steelhead, chinook and sockeye salmon with oral radio transmitters and VI tags. Tracking test groups through the lower Columbia River hydropower system with SRX 400 receivers to examine passage efficiency.

University of Idaho 8/1996-5/1997
Moscow, ID 83844-1136

Aquaculture Laboratory Technician
Construct and maintain flow through aquarium system for fish disease trials. Performed basic histology and built in river live cages for graduate student projects.

ORIGINAL

Prince William Sound Aquaculture Corporation 3/1995-9/1995
Wally Neorenberg Fish Hatchery
Whittier, AK 99693

Fish Culture Technician
Performed all aspects of chinook, coho, sockeye, pink, and chum salmon husbandry including harvest of returning adults, stripping and fertilization of eggs, incubation, rearing and feeding of juveniles. Responsible for skiff operation and outboard motor maintenance during collection of spawning adults. Supervised two individuals during the construction of a floating feed barge and general aquaculture related maintenance projects around the hatchery.

Peer Reviewed Publications:

Cavender WP, Johnson KA, Cain KD (2003) Distribution of *Myxobolus cerebralis* within a free-flowing river system during the migration period for juvenile anadromous salmonids in Idaho. Journal of Aquatic Animal Health 15:158-166

Cavender WP, Wood JS, Madison PS, Overturf K, Cain KD (2004) Real-time quantitative polymerase chain reaction (QPCR) to Identify *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 60:205-213

Wagner, WJ, Arndt, RA, Billman, EJ, Forest AM, and Cavender, WP (2008) Comparison of the Efficacy of Iodine, Formalin, Salt, and Hydrogen Peroxide for Control of External Bacteria on Rainbow Trout Eggs. North American Journal of Aquaculture 70: 118-127

References:

Drew Cushing (Aquatic Chief)
Utah Division of Wildlife
1594 West Temple, Suite 2110
Salt Lake City, Utah 84114-6301
Email: drewcushing@utah.gov
Office Phone: 801-538-4774
Cell Phone: 801-230-6119

Dr. Kenneth Cain (Associate Professor Fisheries Science/Aquaculture)
University of Idaho
Department of Fish and Wildlife Sciences
Moscow, Idaho 83844-1136
Email: kcain@uidaho.edu
Office Phone: 208-885-7608
Cell Phone: 208-669-1292

Deedra Hawk (Wildlife Forensics and Fish Health Laboratory Director)

ORIGINAL

Wyoming Game and Fish Department
Biological Science Building Room 322E
1000 E. University Ave.
Laramie, Wyoming 82071
Email: DHawk@uwyo.edu
Office Phone: 307-766-5618
Cell Phone: 307-761-1124

Vicki Milano (Fish Health Laboratory Director)
Colorado Parks and Wildlife
122 East Edison
Brush, Colorado 80723
Email: Vicki.Milano@state.co.us
Office Phone: 970-842-6308
Cell Phone: 970-370-3015

ORIGINAL

Maria L. Hansen

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mariahansen@utah.gov

Education

University of Hawaii	2005	Microbiology	MS
Brigham Young University Hawaii	2001	Biology	BS

Experience

Microbiologist, Division of Wildlife Resources

Fisheries Experiment Station- Logan, Utah 2014-Present

I conduct routine health examinations of Utah State hatchery, and wild fish. I am able to perform assays in the parasitology, bacteriology and virology sections which are outlined in the American Fisheries Society Blue Book and report results to the fish pathologist. I assist with diagnostic case necropsies and perform research studies in order to further the fish health program within the Division. I am involved in the accreditation program for the lab and author standard operation procedures needed to help to develop a quality program for FES. I am appointed to the quality assurance coordinator for the laboratory. These duties include implementation and monitoring of the quality program and assuring compliance with quality control, policies, procedures, validations and calibrations. I am responsible for identifying problems within the quality system and recommending any training, revision, corrections and improvements to the system. I am also involved in outreach both within the Division as well as among local universities.

Molecular, Bacteriology, Immunohistochemistry and Parasitology Laboratory Technician

Utah Veterinary Diagnostic Laboratory- Logan, Utah 2009-2014

I am responsible for all diagnostic testing in the microbiology, immunohistochemistry and parasitology laboratories. I also work in molecular, serology, pathology, and histology departments at the UVDL. I receive samples and complete diagnostic testing in these areas, then report results to the veterinary pathologists and in our laboratory information management system. I commonly work at biosafety levels 1 and 2, and am proficient in the personal protective equipment needed to complete duties safely therein. I am required to dispose of chemical and biohazard waste. I routinely perform aerobic and anaerobic cultures, salmonella and egg culture, abortion screens, fecal cultures, fungal cultures, milk cultures, campylobacter culture, antibiotic susceptibility testing using standard plate method as well as MIC plate method. I use the API and Crystal BBL Identification systems as well as identification using

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biochemical reactions and morphology. I am formally trained and proficient in using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry to quickly and correctly identify microorganisms of various types. I am proficient in making media, pouring plates and slants. I also pack and ship potentially hazardous and infectious materials in compliance with IATA and DOT guidelines.

I am responsible for authoring and implementing standard operating procedures. I am familiar and able to operate machinery such as a hydraulic table and hoist for large animals, a band and air saw, an incinerator and cremains processor. I am able to assist in postmortem examinations and receive and unload large animals. I am able to identify tissues grossly.

I am trained in histological procedures such as trimming in diagnostic tissues, processing, paraffin embedding of tissues, tissue sectioning and coverslipping. Tissue staining including H and E, silver stains, gram stains, trichrome, GMS and differential stains are routinely performed. I attended advanced training in immunohistochemistry at the Biocare Medical headquarters in Concord California.

My parasitology duties include the identification of parasites using fecal floatation methods, impression and fecal smears. I routinely use specialty staining for the identification of parasites as well. I am also responsible for tritrichamonad culturing and reporting.

I am able to perform various molecular diagnostic assays using basic extraction methods from tissues, fluids and some fixed (FFPE) samples, following through to PCR, and/or qPCR. I am familiar with the ABI 7500 fast and Cepheid smart cycler qPCR platforms as well as conventional PCR using the Eppendorf MasterCylcer machine. I am able to analyze the data generated from qPCR as well as prepare and read agarose gels. I am familiar with using the Agilent Bioanalyzer system to compose data generated via electrophoresis, speciation techniques using the melting point of the molecular model as well as qualitative techniques such as the addition of internal controls. I have helped research, develop, and implement many real time techniques as well as multiplexing technology.

I perform Enzyme-Linked Immunosorbent Assays (ELISA) to detect agents of diagnostic importance. I routinely execute the testing and reporting of prion diseases for the state of Utah and surrounding areas. Because of the time sensitive genre of this testing, I greatly understand the importance of accurate and rapid results in a diagnostic environment.

I am actively involved in the laboratory quality program. This involves documenting and validation of all reagents and samples, extensive proficiency testing, and sending out results to be confirmed by other labs. I have authored and implemented several standard operating procedures (SOP) for new assays. Trend tracking and auditing all data is essential to the quality program as is keeping up with new procedures in the field. Attention to detail and making good quality control habits routine are essential to my success in this area. The UVDL was able to achieve full accreditation with the National Veterinary Services Laboratories with this quality system in place.

Microbiology Lab Technician

Gossner Foods-Logan, Utah 2007-2009

ORIGINAL

Duties include microbiological testing of cheese, milk, soup, and juice samples. Basic milk tank screening such as determining somatic cell counts and the testing for the presence of antibiotics is conducted and results are reported to produces. Quality control testing of air and surface area using basic microbiological methods is conducted. Working with others as a group was imperative here.

Microbiology Technician

Board of Water Supply- Honolulu, Hawaii 2005-2007

I performed routine water quality testing of all public water systems and sources throughout the island of O'ahu. I was responsible for collecting water samples from several points daily, returning to the lab and performing microbiological tests using the column filtration method. A general knowledge of water systems and sources was gained. Several outreach programs were also a part of this job. As an employee of BWS I made presentations at local schools about conserving water and Hawaii's natural aquifer. Following company SOP's and data analysis were used daily. I was also able to work with other departments (Chemistry, Hydro-Geology and Plant-Operations) in order to perform my job more efficiently.

Research Assistant

Virology, University of Hawaii- Honolulu, Hawaii 2003-2005

Virology lab work involved maintenance of cell lines (primarily murine and primate), media making, ordering consumables and storing and cataloguing of samples. I infected eggs and cell monolayers for virus propagation and ordered supplies for the lab. I have experience using Immunofluorescent staining and reading stained slides using a UV microscope. Virus propagation and purification using in vivo and in vitro methods was used routinely. Laboratory animal handling certification was obtained.

Laboratory Technician

Molecular Genetics Program –La'ie, Hawaii 2000-2001

I performed DNA extractions from human blood samples. I then was responsible for cataloguing and storing these samples as well as preparing and packing them for shipping.

Research and Grants

Recipient of a research grant at the University of Hawaii from SeaGrant and NOAA. I studied the effects of marine bacteria on the human poliovirus and was able to isolate and identify (using PCR and sequencing) bacteria that have a negative effect on viral replication. This was shown using the plaquing method and cell culture. Results and thesis were presented at the American Society for Microbiology general meeting in 2005 and was also featured on Microbe Radio in the same year.

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As an undergraduate student I was involved with a fish ecology project which included tagging and counting fish in a local lagoon. The health and population of these fish were monitored for 4 consecutive months.

Teaching

As a graduate student, I was responsible for teaching two labs (general biology and cell biology) per semester. In doing so, I learned to prepare lectures, write a syllabus, correct exams, and deliver information via public speaking.

I have been involved both as a participant and as president of a local club and organization. I am comfortable with instructing individuals and presenting to an audience which is requisite in my work for this not-for-profit group.

Member of the American Society for Microbiology 2002-2005

Member of the American Fisheries Society 2014

Certification and Continuing Education

CE- Environmental DNA (eDNA) sampling for freshwater species American Fisheries Society Joint Meeting Grand Junction, CO 2017

CE- Intro to Fish Culture Fisheries Experiment Station Logan, UT 2014

CE- Bacterial Identification using MALDI-TOF Mass Spectrometry Bruker Daltonics Logan, Utah 2014

CE- Imuunohistochemistry using Biocare Intellipath Automated Staining Biocare Medical Concord, CA 2012

ORIGINAL

CHRISTOPHER J. HECK

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North Logan, UT 84341
Home (435)752-2654
Work (435)752-1066 ext. 25

CURRENT EMPLOYMENT

Utah Division of Wildlife Resources, Fisheries Experiment Station Logan, Utah
(2009-current) Title: Virologist.

- Cell culturist/virologist for DWR fish health inspections at state hatcheries, wild broodstock operations and for diagnostic investigations. Utilize PCR and Electron Microscopy to confirm diagnosis of viral infection.
- Conducted research on pilot scale production of Flavobacterium psychrophilum outer membrane prep vaccine for field trials. Utilized 5L and 100L fermenters and pilot scale Tangential Flow Ultrafiltration to produce vaccine.
- Maintain and develop standard operating protocols (SOP) for molecular diagnostic equipment and train personnel in molecular biology techniques.

EXPERIENCE

Utah Veterinary Diagnostic Lab
Research Associate (2006-2009).

- Conducted research on developing an automated QPCR assay for Caprine Encephalitis and Arthritis Virus. Extracted DNA and RNA, sequenced amplicons, developed standards for QPCR and set up robot for extraction and plate setup. Virus isolation using cell culture and egg based culture.
- Conducted research on new methods to control virulence of Edwardsiella ictaluri in catfish. Set up bioassays to detect quorum sensing molecules and used SPME and HPLC/HPLC-MS to identify Q.S. molecules of interest.
- Managed Electron Microscopy Lab. Used TEM and negative staining techniques to detect and identify viral pathogens of veterinary interest. Used TEM and thin section/staining to identify pathognomonic effects in various tissues. Utilized FE-SEM to research effects of implantable devices on tissue and bone and identify spore stages of a parasitic fish pathogen.

Utah Division of Wildlife Resources, Fisheries Experiment Station Logan, Utah (2004-2006)
Title: Bacteriologist

- Bacteriologist for DWR fish health inspections at Utah state hatcheries, wild broodstock operations, and for diagnostic investigations. Applies standard microbiological procedures, methods, and instrumentation for the analysis of samples for laboratory diagnosis of diseases in fish.
- Oversee transport of biologicals to FES and/or other labs for processing in accordance with Blue Book standards outlined by the American Fisheries Society.
- Prepares collected and submitted samples for diagnostic testing including biochemical, histochemical, and immunohistochemical tests for determination of prohibited pathogens.
- Extracts DNA from submitted samples and develops, performs and troubleshoots PCR analysis for suspected pathogens.

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- Developed protocol and methodologies for production and formulation of a subunit vaccine against Bacterial Coldwater Disease. Conducted test to determine efficacy of vaccine preparation.
- Responsible for training of lab personnel in various molecular techniques; responsible for developing Standard Operating Procedures (SOP).
- Maintains technical data, generates reports and findings electronically. Maintains quality control/quality assurance measures and report laboratory findings in a timely fashion.
- Maintains and troubleshoots microscopes, cameras, incubators, thermocyclers and other laboratory equipment. Responsible for identifying and purchasing lab equipment.
- Assists with virology and parasitology labs.

Utah State University Biotechnology and Genomics Research Center (1997 - 2004)
Manager - Fermentation/Bioprocessing Facility
Research Associate - Mass Spectrometry Lab

- Lead person for planning and implementation of the new Fermentation/Bioprocessing Facility. Expanded and upgraded capabilities of the facility by acquiring used equipment and rebuilding/refurbishing existing equipment.
- Operated and maintained all equipment of the Fermentation/Bioprocessing Facility and trained operators and lab personnel to properly and safely use equipment as needed.
- Developed and/or implemented methods for inoculation, growth, screening, and storage of microbes producing desired products. Responsible for purification, analysis and QA/QC of proteins, peptides and microbial products.
- Responsible for the organization, documentation and record keeping of all work performed.
- Responsible for research design, conduct of experiments, analysis of results, and preparation of interim reports for clientele contracting with the Facility.
- Taught sections of USU CHEM 612 (protein purification class). Responsible for creation of section content, text and lab materials. Conducted lectures and laboratory sessions.
- Laboratory instructor for Industrial Training Programs in Fermentation, Protein Purification and Animal Cell Culture. Responsible for creation of section content, text and lab materials. Conducts lectures and laboratory sessions.

USU Department of Biology. Research Technician/Lab manager. 1995-1997. Isolation and characterization of retinal gap junction proteins and cell adhesion molecules. Utilized immuno-electron microscopy, cryo sectioning, confocal microscopy, cell culture and protein purification techniques. Co-authored papers and grant proposals, trained lab personnel, managed lab operations

BIO/WEST Inc., Biologist. Logan UT. 1991-1993. Conducted surveys of endangered fish species and water quality for Grand Canyon EIS. Stream survey crew leader for EA and NEPA permitting in northern Idaho. Consulted on bioremediation pilot studies.

USU Department of Biology. Research Technician/Lab manager 1989-1991. Conducted research on host/cell interactions involving biocontrol agents of plant pathogens. Initiated research on microbial inoculants for metal-contaminated soils. Utilized biochemical and molecular techniques to generate mutants, determine biological activity and characterize lectin cell recognition molecules. Co-authored papers and grant proposals, presented at national scientific meetings, trained lab personnel, managed lab operations.

ORIGINAL

Univ. of North Carolina/Chapel Hill School of Medicine. Research Biologist. 1987-1989. Conducted research on the purification and characterization of peptide neurohormones. Utilized radio-immunoassays, peptide radio-labeling, electrophoresis and liquid chromatography. Made and purified polyclonal antibodies in rabbit and goat. Isolated neuroactive enzymes from pig and rat pituitaries.

Western State College. Departments of Biology and Chemistry. Graduate Research Assistant. 1985-1987. Conducted research on the purification and characterization of isoenzymes involved in plant development. Conducted research on root colonizing microbes associated with metal-resistant plants used in mined land reclamation.

Currecanti County, CO.Contract Water Quality Technician. 1985-1987. Performed organic, inorganic and biological water quality analysis for county, city of Gunnison and Currecanti National Monument.

University of Minnesota Soil Science Department. Laboratory Assistant.. 1982-1984. Assisted principal investigator and graduate students with laboratory, greenhouse and field research for an applied soil microbiology project. Growth and preparation of bacteria for soil inoculants. GC analysis of nitrogen fixation.

EDUCATION

Master of Arts and Sciences, Western State College, Gunnison, CO - 1989. Biology
Bachelor of Science, Univ. of Minnesota, Saint Paul. 1985. Soil Science/Microbiology

ORIGINAL

CHRISTINE SWAN

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EMPLOYMENT HISTORY

State of Utah, Fisheries Experiment Station 1/1/2007 — present

1465 W 200 N, Logan UT

- Program supervisor and laboratory manager for Utah's fish pathology/diagnostic laboratory.
- Provide a comprehensive program of fish health inspection and certification services for State fish hatcheries and free ranging fish populations.
- Schedule and perform inspections for State hatcheries and wild/feral populations and oversee the collection and transport of samples to the Fisheries Experiment Station for fish health testing in accordance with Utah State legal requirements and Blue Book Standards as defined by the Fish Health Section/American Fisheries Society.
- Supervise a team of 3 microbiologists and 1 technician through the process of fish health testing using laboratory methods that include necropsies, cell culture, biochemical analysis, polymerase chain reaction analysis (PCR), histopathology, antibiotic sensitivity testing and fluorescent antibody testing to identify viral, bacterial and parasitic fish pathogens.
- Schedule and supervise the collection and examination of samples for Utah's Whirling Disease survey using PCR analysis to monitor parasite distribution.
- Provide diagnostic and troubleshooting services for State fish hatcheries and wild/feral fish populations throughout the State.
- Work with the Fish Pathologist/Station Director to provide therapeutic treatment recommendations in accordance with state and federal guidelines for safe and effective legal use of approved compounds.
- Provide research to address fish health issues such as vaccine development, improved treatment methods and best management practices with an emphasis on infectious disease monitoring and prevention.
- Work with hatchery personnel and provide training on disinfection strategies, disease prevention and best management practices.
- Assist and advise in the development of policies and procedures relative to fish culture, fish health and permitting for the movement of live fish and gametes within and across State borders
- Work and correspond with outside sources including universities and state and federal agencies to provide solutions to fish health and aquaculture related issues.
- Attend and give presentations during professional meetings.
- Provide annual summary reports and updates to Utah's Fish Health Policy Board and Division of Wildlife aquatic staff.
- Prepare reports, research and funding proposals, maintain annual budgets and perform annual employee evaluations.

State of Utah, Fisheries Experiment Station 1/1/2007 — present

1465 W 200 N, Logan UT

- Schedule and perform fish health inspections for State of Utah hatcheries and wild/feral populations to establish and maintain disease certifications.
- Collect and transport virology, bacteriology and parasitology samples for fish health inspections.
- Perform necropsies and process and evaluate biological samples for fish health inspections and diagnostic investigations.
- Submit written and verbal laboratory reports to the Fish Health Specialist and State Fish Pathologist.
- Laboratory techniques including DFAT, ELISA, bacterial culture, identification and sensitivities, SDS-Page, fluorescent and light microscopy, cell culture, PCR, PTD.
- Develop and maintain protocols and SOPs. Prepare and practice appropriate QA/QC measures.

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- Prepare *Flavobacterium psychrophilum* vaccine preparations (oral and injectible) and carry out laboratory safety, efficacy and challenge trials. Move this vaccine to field situations and inject over 15,000 brood fish annually.
- Work with State Fish Pathologist and Fish Health Specialist to diagnose disease outbreaks. Perform examinations, culture and identify bacteria and other pathogens.
- Work with University of Idaho on probiotic and attenuated vaccine field trials.
- Work with hatchery personnel on preparation of probiotic feeds, feeding strategies and safety measures.
- Instruct and schedule microbiologists, technicians and hatchery personnel to effect large scale brood stock vaccinations against *F. psychrophilum*.
- Maintain Aquatic Animal Inspector Certification by completing continuing education requirements.
- Stay current on new research and maintain contact with fish health professionals.

Research Assistant/Aquaculture Graduate Teaching Assistant/MS Student, University of Idaho, Ken Cain 6/1/2003 — 12/1/2006

5th and Line Street, Moscow, ID

- Used ELISA, cell separations, tissue culture and skin explants for the Identification of a localized mucosal immune response separate from the systemic response in a teleost fish after anal or intraperitoneal immunization with various preparations of either Flavobacterium psychrophilum or a protein-hapten carrier.
- Characterized immunoglobulin M and other proteins from serum and mucus of rainbow trout utilizing SDS-PAGE, liquid chromatography tandem mass spectrometry, tryptic digestion, high performance liquid chromatography and internal sequencing. Work with protein assays, protein-hapten conjugation, leukocyte isolation, enumeration and culture, western blot, visualization with silver, sypro ruby and coomassie stains, PDVF membrane transfer, B-cell staining, in vitro incubation of tissues for antibody production, vaccine preparation, sample dialysis and concentration, media and reagent preparation and supplies requisition.
- Taught and advised 25 students in aquaculture lab including system set up, water quality, fish stocking, and production planning, occasional class lecture, graded assignments, tests, quizzes, laboratory notebooks and presentations.

Legal Assistant, Frank and Rosen 5/1/1991 — 7/1/1998

1200 Hoge Bldg., 705 2nd Avenue, Seattle, WA

- Executive legal secretary/assistant for senior partner, legal transcription, word processing of pleadings and correspondence, litigation files management, legal research, court filings, interviewing, training, client contact.

EDUCATION

- **University of Idaho** Moscow, ID
  Master's Degree 08/2006
  GPA: 3.82 of a maximum 4.0
  Credits Earned: 61 Semester hours
  Major: Fishery Resources emphasis on Fish Health
  Relevant Coursework: Biochemistry, Current Issues in Fish Health, Immunology, Statistical Analysis, Fish and Wildlife Seminar, Molecular Cell Biology, Sustainable Aquaculture, Virology, Teaching Assistantship
- **University of Idaho** Moscow, ID
  Bachelor's Degree 05/2003
  GPA: 3.87 of a maximum 4.0
  Credits Earned: 63 Semester hours
  Major: Fishery Resources **Honors:** Summa Cum Laude
  Relevant Coursework: Microbiology, Fish Health Management, Ichthyology, Anatomy and Physiology, Interdisciplinary Natural Resource Planning, Genetics, Concepts in Aquaculture, Natural Resource Ecology, Limnology, Fisheries Management, Statistics, Ethics, Fish Ecology, Fish and Wildlife Population Ecology, Wetlands Ecology, Principles of Population Dynamics, Society and Natural Resources, Watershed Management
- **Green River Community College** Auburn, WA
  Three Associate's Degrees and a pre-professional certificate 05/2001

ORIGINAL

GPA: 3.74 of a maximum 4.0
Credits Earned: 186 Quarter hours
Major: Water Quality **Honors:** Magna Cum Laude
Major: Parks Management **Honors:** Magna Cum Laude
Major: Forest Resources **Honors:** Magna Cum Laude
Major: Forest Resources -- Preprofessional **Honors:** Magna Cum Laude
Relevant Coursework: Chemistry, Science &Business Writing, Biology, Animal Biology, Organic Chemistry, Biochemistry

JOB RELATED TRAINING

- Flavobacterium Workshop - Flavobacterium conference Auburn, AL. 10/2015
- AFS Fish Health Section Meeting, Port Townsend, WA. 6/2013
- Western Fish Disease Workshop, Boise ID. 6/2012
- Aquatic Pharmacology WATS 6900 3.00 GPA hours, 4.00 GPA 5/2008
- Flavobacterium Workshop - Flavobacterium conference Leetown WV 5/2007
- AFS Fish Health Section - Nutrition, "Fish Nutrition". 6/2006
- AFS Fish Health Section - Virology, "Regional Fish Virus Information". 7/2003
- Group Dynamics - "New ways to work with coworkers". 2000

CERTIFICATION

- Aquatic Animal Health Inspector, American Fisheries Society, April 2013

PROFESSIONAL PUBLICATIONS

- Cain, K., Swan, C. (2011) Barrier function and immunology: "the multifunctional gut of fish" (invited book chapter). In: Fish Physiology, Vol 30 (ed. by M. Grosell, A.P. Farrell, C.J. Brauner), pp. 111-134. Elsevier Academic Press, San Diego.
- Swan, C. M., Lindstrom, N. M. and Cain, K. D. (2008), Identification of a localized mucosal immune response in rainbow trout, Oncorhynchus mykiss (Walbaum), following immunization with a protein-hapten antigen. Journal of Fish Diseases, 31: 383–393. doi: 10.1111/j.1365-2761.2008.00918.x
- Drennan, J.D., LaPatra, S.E., Swan, C.M., and Cain, K.D. (2007), Characterization of serum and mucosal antibody responses in white sturgeon (Acipenser transmontanus Richardson) following immunization with WSIV and a protein hapten antigen. Fish and Shellfish Immunology, 23: 3: 657-669.

ORIGINAL

Danielle Van Vliet

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dvan@utah.gov

Education

Michigan State University May 2011
Department of Fisheries and Wildlife
Bachelors of Science

Michigan State University May 2016
Department of Fisheries and Wildlife
Aquatic Animal Health Laboratory
PhD

Professional Presentations

The 2012 American Fisheries Society- Fish Health Section Annual Meeting
"Prevalence of Bacterial Cold Water Disease within Michigan State Fish Hatcheries from 2005-2011"
Danielle Van Vliet, T.P. Loch, A. Diamanka, and M. Faisal
La Crosse, Wisconsin Summer 2012

38th Annual Eastern Fish Health Workshop
"An update on Bacterial Cold Water Disease in Michigan"
Danielle Van Vliet, T. P. Loch, and M. Faisal
Gettysburg, Pennsylvania Spring 2013

MSU Fisheries and Wildlife GSO Symposium
"Assessment of risks posed by *Flavobacterium psychrophilum* conservation efforts in the Great Lakes"
Danielle Van Vliet, T. P. Loch, and M. Faisal
East Lansing, Michigan Spring 2014

39th Annual Eastern Fish Health Workshop
"Molecular epidemiology of *Flavobacterium psychrophilum* isolates from feral and hatchery-reared *Oncorhynchus* spp. (Family Salmonidae) of the Great Lakes Basin"
Danielle Van Vliet, T.P. Loch, P. Nicolas, and M. Faisal
Shepherdstown, West Virginia Spring 2014

Great Lakes Fish Health Committee Meeting
"Antibiotic susceptibility profiling of Great Lakes basin *Flavobacterium psychrophilum* isolates"
Danielle Van Vliet, T.P. Loch, and M. Faisal
East Lansing, Michigan Spring 2016

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Professional Presentations (cont'd)

Great Lakes Fish Health Committee Meeting
"Antibiotic susceptibility profiling of Great Lakes basin *Flavobacterium psychrophilum* isolates"
Danielle Van Vliet, T.P. Loch, and M. Faisal
East Lansing, Michigan — Spring 2016

American Fisheries Society- Utah Chapter Meeting
"Rainbow trout strain hybridizations as a management strategy to control Bacterial Cold Water Disease"
Cristi Swan, Eric Wagner, Maria Hansen, Danielle Van Vliet, Wade Cavender
Grand Junction, Colorado — Spring 2017

The 2072 American Fisheries Society- Fish Health Section Annual Meeting
"Antimicrobial Minimum Inhibitory Concentrations of *Flavobacterium psychrophilum*"
Danielle Van Vliet, T.P. Loch, P. Smith, and M. Faisal
East Lansing, Michigan — Spring 2017

Publications

Van Vliet, D., T.P. Loch, M. Faisal. 2015. *Flavobacterium psychrophilum* infections in salmonid broodstock and hatchery-propagated stocks of the Great Lakes basin. Journal of Aquatic Animal Health, 27: 192-202.

Van Vliet, D., G.D. Wiens, T.P. Loch, P. Nicolas, M. Faisal. 2016. Genetic diversity of *Flavobacterium psychrophilum* isolated from three *Oncorhynchus* spp. in the U.S.A. revealed by multilocus sequence typing. Journal of Applied and Environmental Microbiology, 82: 3246-3255.

Van Vliet, D., T.P. Loch, P. Smith, M. Faisal. 2017. Antimicrobial susceptibilities of *Flavobacterium psychrophilum* isolates from the Great Lakes basin, Michigan. Microbial Drug Resistance, *ahead of print*.

Research Experience

Graduate Research Assistant — Concurrent throughout graduate career
Michigan State University — 2011-2016
Aquatic Animal Health Laboratory
East Lansing, Michigan

Fish Health Microbiologist — May 2016- present
Utah Division of Wildlife Resources
Fisheries Experiment Station
Logan, Utah

ORIGINAL

Teaching Experience

Graduate Student Evaluator
Michigan State University
University Undergraduate Research and Arts Forum — Spring 2013

Guest Judge and Evaluator
Michigan State University
University Undergraduate Research and Arts Forum — Spring 2014

Graduate Teaching Assistant
Michigan State University
College of Natural Science-Biological Sciences Program
BS172- Organismal Biology Lab — Spring 2015

Graduate Teaching Assistant
Michigan State University
College of Natural Science-Biological Sciences Program
BS172- Organismal Biology Lab — Summer 2015

Graduate Teaching Assistant
Michigan State University
College of Natural Science-Plant Biology/Integrative Biology
ZOL355L- Ecology Lab — Fall 2015

Guest Judge and Evaluator
Flint Regional Science Fair-Senior Division
Flint, Michigan — Spring 2016

Certifications

The World Organization for Animal Health: Understanding the OIE
American Fisheries Society — Spring 2012

Molecular Diagnostic Tests for Aquatic Animal Pathogens Continuing Education Course
American Fisheries Society — Summer 2012

Approaches and Considerations for Proper Fish Welfare
American Fisheries Society — Spring 2013

Fish Pharmacology and Treatment
American Fisheries Society — Spring 2014

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Skills

- Extensive work with fish necropsy and clinical pathology
- Extensive work with standard bacteriological culture techniques (including media preparation, biochemical test inoculation and interpretation, bacterial identification)
- Extensive work with standard fish parasitological techniques and gross pathology
- Antibiotic sensitivity applications
- Molecular diagnostic techniques (PCR and qPCR)
- Phylogenetic techniques and associated software (Multilocus Sequence Typing, eBURST, MEGA, BioEdit, Mr. Bayes)
- Statistical analysis of data and associated software (Excel, R, and SAS)
- Organizational and management skills of undergraduate research assistants
- Teaching hands-on biology and ecology skills to undergraduate students
- Managing undergraduate students in a laboratory-learning setting

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Chemical Hygiene Plan
Fisheries Experiment Station, Utah Division of Wildlife Resources
(Updated March 2017)

INTRODUCTION

The Fisheries Experiment Station Chemical Hygiene Plan was developed to meet requirements of the Occupational Safety and Health Administration standard on "Occupational Exposures to Hazardous Chemical in the Laboratories", CFR 1910.1450. The standard is known as the "Laboratory Standard".

Many laboratory chemicals are hazardous. However, if used properly in adequate facilities and with appropriate personal protective equipment, they may be used safely. The Chemical Hygiene Plan establishes procedures, equipment, and work practices to protect laboratory employees from health hazards presented by hazardous chemicals. Employees using the laboratory must be familiar with chemicals used in the lab, containment and storage equipment and emergency procedures. To accomplish this, a laboratory safety tour needs to be completed by each employee that uses or works within the laboratory.

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Chemical Hygiene Plan

1. RESPONSIBILITIES

Each individual using chemicals is responsible for awareness of hazardous characteristics and safe use of the product.

1.1 Chemical Safety Committee

The chemical safety committee is composed of three Fish Health Technologists and laboratory supervisor. The committee is responsible for reviewing and recommending laboratory policy on the use, storage and disposal of chemicals and to review and make recommendation on lab policy concerning other non-biological hazards.

1.2 Chemical Hygiene Officer

The chemical hygiene officer (Lead Technologist) is responsible for daily operation of chemical hygiene. Specifically, the individual will

1. Review and update the Chemical Hygiene Plan as needed.
2. Develop and conduct training and information programs on laboratory safety to promote safe handling of hazardous materials.
3. Provide technical assistance to investigators and laboratory staff on issues of laboratory safety.

4. Review application of chemical safety practices and procedures through periodic evaluation lab and safety equipment such as eye wash stations, safety showers and fume hoods.
5. Maintain knowledge of Federal, State and Local regulations concerning hazardous materials use and disposal.
6. Manage disposal of hazardous materials and develop methods to minimize hazardous waste.

1.3 Laboratory Supervisor

The laboratory supervisor is responsible for chemical safety in the laboratory, and specifically:

1. To be aware of hazards associated with chemicals used and to inform laboratory staff of these hazards.
2. Select and employ lab practices and controls to minimize the potential for exposure to hazardous materials.
3. Ensure that laboratory staff receives lab safety training and employs practices and procedures identified in the standard operating procedures of each protocol.
4. Ensure staff using lab facility maintains records with the Chemical Hygiene Plan.

1.4 Laboratory Employees/Staff Using the Laboratory

Employees using the laboratory are responsible for safe use of chemicals in laboratories and following prescribed practices and procedures to minimize risk, as well as, being aware of information in the Material Safety Data Sheets for chemicals used in lab studies or procedures. In addition, the employee shall immediately report to the Laboratory Supervisor all facts pertaining to accidents involving potential exposure to hazardous materials.

It is imperative that individuals using chemicals in reactions be aware of hazards of the individual chemical reactions as well as those of reacted products.

2. GENERAL LABORATORY SAFETY PROCEDURES

Laboratories contain many potential hazards for the untrained and uninformed individual. The hazards may include toxic chemicals, infectious material, hot surfaces, electric current, glassware, systems under pressure, spinning devices, extremely cold items or a combination of these. With proper training and an understanding of practices to reduce exposure to these materials, employees can perform assigned tasks with minimal health risk. While certain laboratory protocols require extensive safety precaution, most can be performed safely by observing these general procedures:

1. Thoroughly wash your hands with soap and water to prevent ingestion of harmful materials before handling food or drink.
2. Mouth pipetting is strictly prohibited.
3. Use proper personal protective equipment such as gloves, safety glasses, goggles, lab coats, etc. Contact lenses should not be worn when working with volatile chemicals.

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Proper use of MSDS forms will help an individual decide on the type of personal protective equipment to use.

4. Do not smell or taste chemicals.
5. Know the location of the nearest fire extinguisher and the siren from the fire alarm.
6. Place broken glass or other sharp objects in puncture resistant containers.
7. Dispose of chemicals properly.
8. Store chemicals properly and minimize quantities handled.
9. Use a laboratory fume hood when working with volatile compounds.

To summarize these general procedures, maintain good person hygiene, store materials properly, be familiar with chemical characteristics and use adequate personal protective equipment and engineering controls. Employing these "common sense" procedures will help achieve a safe and productive work area.

2.1 EATING, DRINKING, SMOKING IN LABORATORIES

Eating, drinking, smoking, gum chewing and taking medicine in laboratories where hazardous chemicals are used should be strictly prohibited. Food, beverages, cups and other drinking utensils should not be stored in areas where hazardous chemicals are handled or stored. Glassware used for lab operations should never be used to prepare or consume food or beverages. Laboratory refrigerators, ice chests, ovens and so forth should not be used for food storage or preparation. Lab water sources and deionized lab water should not be used for drinking water.

3. SAFETY DATA SHEETS

3.1 General

The Occupational Safety and Health Administration (OSHA) requires chemical manufacturers and importers to develop or obtain a Safety Data Sheet (SDS) for each chemical they produce or import. SDS's contain information about the chemical, its physical and health hazards and other health and safety data. SDS's for chemicals used in this facility can be found online at http://www.msds.com/

3.2 Description

All SDS's have specific sections that contain standard information about the chemical. A short description of the sections and type of information about the chemical follows:

The Safety Data Sheet must include the following information:

- Identity the substance designated on the container label.
- Physical and chemical characteristics of the hazardous chemical.
- Physical hazards
- Known acute and chronic health effects and related health information.
- Primary routes of entry in the body.
- Information on exposure limits.

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- Whether a hazardous chemical is considered a carcinogen by OSHA, the International Agency for Research on Cancer, or the National Toxicology Program.
- Precautions for safe handling.
- Generally acceptable control measures (engineering controls, work practices, personal protective equipment).
- Emergency and first aid procedures, including a 24 hour manufacturer phone contact.
- Date of SDS preparation or most recent change.
- Name, address, and phone number of the party responsible for preparing and distributing the SDS.

4. CHEMICAL USE AND HANDLING

Chemicals are essential components of laboratory research. Used properly, they present minimal physical and health hazards to personnel and the environment. Of critical importance is minimizing the quantities used; therefore reducing the amount that must be purchased and disposed. The cost to dispose partially used products can nearly equal or exceed the initial purchase cost. By using micro techniques, much smaller volumes of reagents are needed which require fewer products to be purchased and stored.

4.1 Container Labels

Manufacturers, importers and distributors must label all containers of hazardous chemicals. Containers include items such as bags, barrels, bottles, etc. The label will often indicate how to store and handle the chemical, what protective clothing you should wear and other safety procedures.

4.1.1 Primary Label

The label must contain the, 1) chemical name, 2) the hazard warning and 3) the name and address of the manufacturer or importer. Hazard warnings may be written or be presented by symbol or both and include terms such as flammable, corrosive, irritant, oxidizer, etc. When containers are received, the labels shall remain intact until the container is empty and discarded.

4.1.2. Secondary Container Labels

Frequently, hazardous chemicals are poured into smaller container from bulk bottles. Each secondary container must have a label that contains, at a minimum, the following information: name of chemical, date of transfer, name or initials of person conducting the transfer and hazard warning terms that appear on the primary container.

4.2 Minimize

The American Chemical Society advocates a “less is better” philosophy of hazardous waste management. It is based on reducing the amount of chemicals that may become waste. This can be done by either eliminating a specific chemical or reducing the quantity used.

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To effectively reduce the amount of hazardous wastes produced in a laboratory, it is prudent to consider: 1) purchasing smaller quantities, 2) how the chemical is used, 3) alternate non-hazardous chemicals and 4) using smaller quantities of chemicals required in assays which should directly influence quantities purchased and stored. Smaller quantities purchased result in less unused chemicals being stored and reduce the potential for chemical exposure to personnel. Extended storage of unused chemicals increases the risk of accidents.

5. CHEMICAL STORAGE

To reduce risk in event of an accident or fire, the quantity of chemicals stored in a laboratory must be kept at a minimum, consistent with needs of the investigator and fire codes. As indicated in Section 4, all primary and secondary storage containers must be labeled to identify the chemical, its manufacturer or importer and the hazard warning.

5.1 Chemical Storage Method

Store chemicals by hazard classification (e.g., oxidizer, combustible, corrosive, unstable, water reactive, etc.) rather than alphabetical order in cabinets or on open shelving not higher than eye level. To determine the chemical hazard class, check the label for hazard information or consult the SDS. The hazard class is listed on the original container.

Once chemicals are segregated by hazard class, compatible classes can be stored together. Incompatible classes must be physically separated from each other. Separation of chemical groups can be by different shelves within the same cabinet; providing secondary containment is used to retain materials should the primary container be breached. Storage areas and containers should be inspected periodically for signs of deterioration. Several of the major chemical firms have developed systems using a color code to define the groups which should be stored together. However, NOT all companies use the same system; therefore, care must be taken when segregating chemicals.

5.2 Refrigerators

Flammable, toxic and uncapped chemicals should not be stored in standard household refrigerators because there is inadequate ventilation which allows explosive mixtures or toxic concentrations to build up. Sparks from light, thermostat and fan switches may be ignition sources for flammable and combustible vapors. Also, laboratory workers generally place their face in the refrigerator when looking for samples, increasing the likelihood of inhaling unvented vapors. Frost-free refrigerators generally contain a drain or hole that carries liquids to a drain pan near the compressor. A leaking flammable substance could end up adjacent to the spark source, the compressor.

All chemicals in a refrigerator must be labeled and no food or drink may be stored in a refrigerator with chemicals.

There are three types of lab refrigerators that reduce the risk of ignition of flammable vapors:

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1. “Explosion Proof” refrigerators that are required only where there is risk of ignition on the inside and outside of the unit.
2. Explosion Safe or lab safe refrigerators and freezers are designed to eliminate ignition of vapors inside the storage compartment by sources also within the environment.
3. Although not considered optimum protection, it is possible to modify domestic refrigerators to achieve some degree of protection. However, the modification process can be applied only to manual defrost units; the self-defrosting models cannot be successfully modified to provide even minimum safeguards against vapor ignition. The minimum procedures for modification include:

a) Relocation of manual temperature controls to the exterior of the storage compartment and sealing all points where capillary tubing or wiring formerly entered the storage compartment.

b) Removal of light switches and light assemblies and sealing of all resulting openings.

c) Replacement of positive mechanical door latches with magnetic door gaskets.

Note: these modification techniques are suggested primarily for domestic refrigerators that will be used for storing chemicals. Not all of these modifications are necessary for refrigerators that will hold biological or nonchemical samples; or more modifications may be necessary.

5.3 Environmental Rooms (Cell Culture Room)

Environmental rooms have the inherent property of being closed air-circulation systems. Thus, the release of any toxic substance in these areas poses potential danger. The cell culture room is a positive air displacement system where air comes into that room, and is not circulated air from the rest of the lab.

6. CHEMICAL DISPOSAL

The Fisheries Experiment Station is bound by a variety of federal, state and local regulations to dispose of hazardous chemicals in a safe, environmentally sound manner. Therefore, the following requirements must be met:

1. **DO NOT** dispose of chemicals or chemical waste in the drains or general trash.

2. Waste alcohols and waste formalin are stored in the “Hazardous Waste” contrainers located in histology.

3. All hazardous waste chemicals must be disposed of through one of two contracted vendors:

Clean Harbors Environmental Service Inc.
2150 North 470 East
Toole UT 84074
801-597-0283
Contract # MA1603

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Veolia North America
709 N Taylor Way
North Salt Lake UT 84054
801-294-7111
Contract # MA1604

All chemicals must be labeled with the chemical name (not the abbreviation or chemical formula or trade name). The containers must have labels identifying the contents. A waste Inventory Form (see attached) is filled out that indicates the chemical name, active ingredients, number and size of containers.

4. Chemicals are to be kept in containers with lids that are impervious to their inherent hazards. The containers must be leak proof and allow headspace for expansion. Do not seal with parafilm. Empty product bottles may be used if the former contents are not incompatible with the waste and old labels are defaced. Solid and liquid forms are kept separate where possible. Contaminated articles such as gloves and absorbent pads constitute hazardous waste by definition.

5. Segregate aqueous from organic wastes and halogenated from non halogenated solvents where possible. (Halogenated solvents contain Fluorine, Chlorine, Bromine, Iodine or Astintine within their composition.) This helps to decrease disposal costs and increase the potential for recycling/reuse/reclamation options.

6. Note when wastes are “used” or “spent”, as opposed to surplus/obsolete virgin products. The latter may be offered for reuse within the laboratory rather than disposed. If used or spent, be sure to note any contaminants in the waste.

7. Empty containers must be completely empty and free from any residual hazards. If the chemical is the sole active ingredient and it appears on the EPA P-listed Waste List, the container must be triple rinsed with an appropriate solvent, collecting the rinsate as hazardous waste. Finally, write the word EMPTY on the face of the label and dispose container to the general trash.

8. Minimize wastes where possible by reducing volume on hand and substituting less hazardous chemicals for hazardous materials. Keep chemical inventories current and offer underutilized chemicals to other facilities.

9. Store chemicals only within distinctly segregated hazard classes, minimizing risk of reactions in the event of a release. Do not store chemical waste where risk of breakage is likely.

10. Sharps, broken glass and other puncture causing items should be disposed of in sturdy, puncture-resistant containers marked Sharps or Broken Glass.

11. Do not accumulate hazardous wastes. Dispose of them regularly.

7. EMPLOYEE INFORMATION AND TRAINING

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The Chemical Hygiene Officer will provide employees using the laboratory with information and training concerning the hazards of chemicals present in the laboratories. Newly hired employees will be trained in laboratory and chemical safety while training of their other job duties is also taking place.

7.1 Informational Requirements

Employees will be informed of or made available:

1. The contents of the Fisheries Experiment Laboratory Protocol Manual.
2. The location and availability of the Chemical Hygiene Plan.
3. What to do in case of exposure or a chemical spill.

7.2 Employee Training

Employee training will include:

1. Methods and observations used to detect the presence or release of a hazardous chemical (such as visual appearance or odor).
2. Health hazards of chemicals and the proper use, and when it is necessary to use the chemical fume hood.
3. Measures employees can take to protect themselves, such as appropriate work practices, emergency procedures and personal protective equipment (PPE).
4. Where the chemical spill kits and filtered face masks are located.

8. Personal Protective Equipment

Personal protective equipment (PPE or PPD's) includes all clothing and other work accessories designed to create a barrier against workplace hazards. These barriers are intended to protect the eyes and face, hands and arms, and body. Laboratory workers must be aware that PPE doesn't eliminate the hazard; if the PPE fails or is improperly used, exposure will likely occur. An assessment of the laboratory environment indicates that in most cases a laboratory coat, gloves and eye protection should be worn during procedures involving wet chemistries or similar manipulations with toxic or corrosive materials.

8.1 Eye and Face Protection

The most likely incident involving the eye or face is a chemical splash. Protection from chemical splashes is attained by wearing safety glasses, goggles or a face shield. Safety glasses are impact resistant, meet flammability test requirements and are of minimum thickness. They offer minimal protection from other than a direct splash. Contact lenses should not be worn in laboratories where chemical splashes may occur or where vapors may be present. In event of chemical splash, it is extremely difficult to remove the contact lens to irrigate the eye due to involuntary spasms of the eyelid. Additionally, gases and vapors may concentrate under the contact lens causing permanent eye injury.

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Face shields cover the eyes, face and throat, providing protection when working with systems under pressure and reactive mixtures. They should always be worn with primary eye protection such as safety goggles. Check SDS's for the type of protection needed. Face shields should always be worn when using LN_2 or the LN_2 tank.

8.2 Hand Protection

Gloves create a barrier between the hand and contact with hazardous materials. Selecting the correct type glove depends on the work practice and chemicals used. Each type of glove material is tested against various chemicals to determine its permeability and break through time. Gloves selected for chemical resistant properties also protect against dry powders.

8.3 Miscellaneous Hand/Arm Protection

Special gloves may be necessary to handle items at extreme temperatures. Select gloves based on their intended use and check their specifications against extreme temperatures to be encountered.

8.4 Protective Apparel

In most instances, a laboratory coat provides adequate barrier protection from minor chemical splashes and contact with other hazardous materials. Lab coats however, are combustible! In situations involving fire, the lab coat as well as other fabrics, such as rayon and polyester may present an additional hazard to the wearer. In such case as a fire, extinguishers and blankets are located throughout the lab.

Work with large quantities of corrosive liquids requires an apron to protect against splashes or a spill. Select aprons that are resistant to solvents, acids and other chemicals.

9. EMERGENCY PROCEDURES

9.1 General

The laboratory may contain hazardous chemicals that may be spilled or released from reactions or as a result of fire. The most common incident involves spilling liquid chemicals from a glass container. Response to the incident depends on the chemical involved, extent of personal injury, and facility damage. Information about the chemical (s) involved is available in the SDS and the identification should be made by the user.

9.2 Initial Procedures

When the incident occurs, take these initial steps:

- Warn others in the area, identifying the chemical if known.
- Use the chemical spill kit appropriate for the chemical spilled
- Contain the liquid or solid with absorbent pads or sheets.
- Extinguish flame/ignition sources if flammable liquid, solid or gas is released.

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- In case of fire, assess the situation before using a fire extinguisher. Dry chemicals from the extinguisher may cause more damage to computer and electronic equipment that a small alcohol or solvent fire.
- Notify the fire department if necessary. Give the following information:
  a. Name
  b. Location of incident
  c. Type of material involved
  d. Name of chemical
  e. How much is spilled
  f. Phone number of caller

*If the chemical has come in contact with:

-Lab coat and/or street clothing, remove these items immediately.
-Skin or eyes, promptly flush the affected area for at least 15 minutes. Use the eyewash or safety shower.

*If the chemical is ingested, rinse out the oral cavity if conscious and seek medical attention.

*Leave the immediate area, staying close enough to control access to the laboratory and relate details to responders.

NOTE: Other initial procedures may be appropriate for specific chemicals. Consult the SDS before use to be aware of recommended spill procedures.

<u>9.3 Follow-up Actions</u>

9.3.1 Injury

Emergency – report to the nearest emergency center (Logan Regional Hospital, 500 E 1400 N, Logan UT 84341) in the event of severe bleeding, head injury, broken bones, respiratory distress or other life threatening injuries. <u>If patient transport or paramedic services are necessary, dial 911.</u>

Non-emergency – If the injury is not an emergency but requires treatment, notify the Administrative Assistant immediately and fill out a workman's compensation form. Schedule an immediate appointment with Intermountain Logan WorkMed at 412 N 200 E, Logan UT 84321, 435-713-2850 and report back to the Administrative Assistant.

NOTE: You may be responsible for all medical expenses incurred if you do not report the injury.

9.3.2 Decontamination and Chemical Waste

Chemical spill cleanup supplies and chemicals are located in the virology lab and in the histology. The main storage area is the Histology room. With the exception of a small volume or quantity of solvents or laboratory reagents, DO NOT ATTEMPT TO CLEAN UP THE SPILL.

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Liquids give off vapors that may be extremely toxic and powders may easily be resuspended by moving around the spill area. Call the local fire department for cleanup assistance. They are trained to wear respiratory protection and have a variety of respirator cartridges available depending on the chemical involved. Chemical waste, including contaminated absorbents and articles, must be prepared and labeled for disposal. Broken glass must be placed in puncture resistant containers for proper disposal, keeping in mind that it is also contaminated, located in histology.

9.4 EMERGENCY PHONE NUMBERS

Fire Station	435-716-9500
Emergency Fire/Medical Services	911
Poison Control Center	1-800-222-1222

Standard Operating Procedure - Ethidium Bromide Waste Management

CAUTION: Ethidium bromide is a mutagen and environmental hazard. It should be handled carefully with gloves, lab coat, and eye protection and disposed of properly.

CAUTION: Ethidium bromide decomposes above 500 F. Autoclaves will not destroy it. Incineration at a regulated medical waste incinerator is recommended as a cost effective, compliant method of disposal. A container for medical waste is available for anything having contact with Ethidium bromide: gels, gloves, paper towels, pipette tips etc. Agarose gels and E-gels should be placed in a zip lock bag and then into the medical waste container.

Used aqueous Ethidium bromide solutions are filtered through an approved charcoal filtration system. This filtering system is then disposed of in the medical waste container.

NOTE: Do not use bleach for decontamination. Bleach converts dyes into a mutagenic compound in the absence of microsomes.

WEAR LAB COAT, GLOVES, AND EYE PROTECTION FOR THESE PROCEDURES.

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FES Waste Inventory Form

	Chemical	Quantity	Unit	No. Containers	Container type
1	Arsenic Compound, Solid	5	Grams	1	Glass Bottle
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
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Biosafety Requirements

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Section IV—Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 2 of this section and discussed in Section 2. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

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Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

A. Standard Microbiological Practices

Initials

✓ 1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

✓ 2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

✓ 3. Eating, drinking, smoking, handling contact lenses, applying

cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

✓ 4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

✓ 5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

✓ 6. Perform all procedures to minimize the creation of splashes and/or aerosols.

✓ 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

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✓ 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

✓ 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

✓ 10. An effective integrated pest management program is required. (See Appendix G.)

✓ 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

None required.

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C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Initials

✓ 1. Special containment devices or equipment, such as BSCs, are not generally required.

✓ 2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

✓ 3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

✓ 4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

Initials

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✓ 1. Laboratories should have doors for access control.

✓ 2. Laboratories must have a sink for hand washing.

✓ 3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

✓ 4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

✓ 5. Laboratories windows that open to the exterior should be fitted with screens.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

A. Standard Microbiological Practices

Initials

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✓ 1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

✓ 2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

✓ 3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

✓ 4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

✓ 5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

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✓ 6. Perform all procedures to minimize the creation of splashes and/or aerosols.

✓ 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

✓ 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

✓ 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

✓ 10. An effective integrated pest management program is required. (See Appendix G.)

✓ 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing

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age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

Initials

✓ 1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

✓ 2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.

✓ 3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

✓ 4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

✓ 5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

✓ 6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

✓ 7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

   a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

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b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

✓ 8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

✓ 9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.

✓ 10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Initials

✓ 1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

✓ 2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with

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hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

✓ 3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

✓ 4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:

a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed

✓ 5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

Initials
CS

✓ 1. Laboratory doors should be self-closing and have locks in

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accordance with the institutional policies.

✓ 2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

✓ 3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

✓ 4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

   b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

✓ 5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

✓ 6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

✓ 7. Vacuum lines should be protected with liquid disinfectant traps.

✓ 8. An eyewash station must be readily available.

✓ 9. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

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✓ 10. HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

✓ 11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.
A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

A. Standard Microbiological Practice

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be

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permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

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8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. (See Appendix G.)

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be

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encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents

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must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.

2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:

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a. Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.

3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

a. Floors must be slip resistant, impervious to liquids, and

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resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory must be sealed.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available in the laboratory.

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9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

b. The laboratory exhaust air must not re-circulate to any other area of the building.

c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

d. HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. A method for decontaminating all laboratory wastes should be

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available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

12. Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the

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primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

A 1. Cabinet Laboratory—Manipulation of agents must be performed in a Class III BSC; and

A 2. Suit Laboratory—Personnel must wear a positive pressure supplied air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices, equipment, and facilities apply to BSL-4.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

3. Mechanical pipetting devices must be used.

4. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Precautions, including those listed below, must be taken with any sharp items. These include:

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a. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

b. Use of needles and syringes or other sharp instruments should be restricted in the laboratory, except when there is no practical alternative.

c. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal or decontamination. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal, located as close to the point of use as possible.

d. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

5. Perform all procedures to minimize the creation of splashes and/or aerosols.

6. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.

7. Decontaminate all wastes before removal from the laboratory by an effective and validated method.

8. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

9. An effective integrated pest management program is required. (See Appendix G.)

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10. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements in accordance with institutional policies.

Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory must be maintained.

While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All persons entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves (as appropriate). All persons leaving the laboratory must take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before

laundering.

After the laboratory has been completely decontaminated and all infectious agents are secured, necessary staff may enter and exit without following the clothing change and shower requirements described above.

2. Laboratory personnel and support staff must be provided appropriate occupational medical services including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired infections.

3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.

5. The laboratory supervisor is responsible for ensuring that laboratory personnel:

a. Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-4 containment.

b. Receive appropriate training in the practices and operations specific to the laboratory facility.

b. Receive annual updates and additional training when procedural or policy changes occur.

6. Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-

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breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside BSL-4 containment unless inactivated by a validated method.

7. Laboratory equipment musts be routinely decontaminated, as well as after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory.

b. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the laboratory. The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.

c. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. Supplies and materials that are not brought into the BSL-4 laboratory through the change room, must be brought in through a previously decontaminated double-door autoclave, fumigation

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chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility. The doors of the autoclave or fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave or fumigation chamber has been operated through a decontamination cycle.

Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the laboratory.

11. Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.

12. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Cabinet Laboratory

1. All manipulations of infectious materials within the laboratory must be conducted in the Class III biological safety cabinet. Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

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The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves.
Class III cabinet gloves must be inspected for damage prior to use and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.
The Class III cabinet must be certified at least annually.

2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower

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area. All protective clothing must be removed in the dirty side change room before showering. Reusable clothing must be autoclaved prior to removal from the laboratory for laundering.

3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eyeglasses must be decontaminated before removal through the personal body shower.

4. Disposable gloves must be worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

Suit Laboratory

1. All procedures must be conducted by personnel wearing a one-piece positive pressure supplied air suit.
All manipulations of infectious agents must be performed within a BSC or other primary barrier system.

Equipment that may produce aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as needed.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's specifications.

2. Workers must wear laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All laboratory clothing must be removed in the dirty side change room before entering the personal shower.

3. Inner disposable gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn

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outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to entering the personal shower. Dispose of used gloves with other contaminated waste.

4. Decontamination of outer suit gloves is performed during laboratory operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the facility must be arranged to ensure sequential passage through an inner (dirty) changing area, a personal shower and an outer (clean) change room upon exiting the room(s) containing the Class III BSC(s).

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on an uninterrupted power supply (UPS).
A double-door autoclave, dunk tank, fumigation chamber, or ventilated airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the door of the cabinet room(s) and the inner change room. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.

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3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

Drains in the laboratory floor (if present) must be connected directly to the liquid waste decontamination system.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment.

Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment. Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.

5. Windows must be break-resistant and sealed.

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6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory.

9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory.
Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes. All HEPA

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filters should be located as near as practicable to the cabinet and laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually. The HEPA filter housings should be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. If BSC exhaust is to be recirculated to the outside, BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a hard ducted, direct connection ensuring that cabinet exhaust air passes through two (2) HEPA filters—including the HEPA in the BSC—prior to release outside. Provisions to assure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.

12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional

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policy.

Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable and airtight and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.

15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

Suit Laboratory

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the

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institutional policies.

Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

Entry into the BSL-4 laboratory must be through an airlock fitted with airtight doors. Personnel who enter this area must wear a positive pressure suit supplied with HEPA filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup.

A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

An automatically activated emergency power source must be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.

2. Sinks inside the suit laboratory should be placed near procedure areas and be connected to the wastewater decontamination system.

3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory, suit storage

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room and the inner change room must be sealed.

Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents must have protection against insect and animal intrusion. Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners should be avoided. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.

5. Windows must be break-resistant and sealed.

6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the BSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.

9. A dedicated, non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation

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systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.
All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings must be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can

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also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.

12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.
Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable, airtight, and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam

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exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.

15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

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Table 2. Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	■ No primary barriers required. ■ PPE: laboratory coats and gloves; eye, face protection, as needed	Laboratory bench and sink required
2	■ Agents associated with human disease ■ Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: ■ Limited access ■ Biohazard warning signs ■ "Sharps" precautions ■ Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: ■ BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ■ PPE: Laboratory coats, gloves, face and eye protection, as needed	BSL-1 plus: ■ Autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 practice plus: ■ Controlled access ■ Decontamination of all waste ■ Decontamination of laboratory clothing before laundering	Primary barriers: ■ BSCs or other physical containment devices used for all open manipulations of agents ■ PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BSL-2 plus: ■ Physical separation from access corridors ■ Self-closing, double-door access ■ Exhausted air not recirculated ■ Negative airflow into laboratory ■ Entry through airlock or anteroom ■ Hand washing sink near laboratory exit
4	■ Dangerous/exotic agents which post high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ■ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ■ Related agents with unknown risk of transmission	BSL-3 practices plus: ■ Clothing change before entering ■ Shower on exit ■ All material decontaminated on exit from facility	Primary barriers: ■ All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	BSL-3 plus: ■ Separate building or isolated zone ■ Dedicated supply and exhaust, vacuum, and decontamination systems ■ Other requirements outlined in the text

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Integrated Pest Management Plan

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State of Utah
Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, Utah 84321

Insect/Rodent Control Plan

A. PURPOSE

The purpose of this document is to describe the programs employed for insect and rodent control at the Fisheries Experiment Station (FES).

B. DEFINITIONS

1. N/A

C. SAFETY PRECAUTIONS

- Any chemicals, traps, barriers or other pest control measures whose use is limited to trained pest control professionals are regarded as dangerous and are handled only by professional pest management personnel.
- If over-the-counter insect/rodent control devices are purchased, the user must read and follow package inserts.

D. EQUIPMENT AND MATERIALS REQUIRED

1. Professional products and methods for pest control issues are provided by Logan Extermination Services, 110 W 1700 S, Logan, UT 84321, (435) 752-8450.
2. Small, seasonal infestations (ants, spiders, etc.) within offices or public use areas of the laboratory can be dealt with by over-the-counter insect control devices or products. If these fail to control infestation, Logan Extermination Services, 110 W 1700 S, Logan, UT 84321, (435) 752-8450, is contacted.

E. PROCEDURES

1. FES has an insect and rodent control contractor who will perform a thorough inspection of building exteriors and provide treatment of infested areas as needed. The specialist is certified by the Utah State Department of Agriculture in pest control and pesticide application.
2. For interior rooms, the FES policy is to prevent and /or control infestations through the use of non-chemical/non-toxic (traps, barriers, etc.) methods wherever possible. When further treatment is needed, the contracted insect and rodent control contractor will perform a thorough inspection of building interiors and provide treatment of infested areas as needed.
3. Pesticides are not applied inside FES without prior notification of administrative personnel; reasonable effort must be made to contact the laboratory manager or station director prior to pesticide application. This precludes application of chemicals that may interfere with diagnostic tests and procedures.
4. If there are insect/vermin issues, FES personnel are to contact the laboratory director or designee.
5. Small, seasonal infestations (ants, spiders, etc.) within offices or public use areas of the laboratory can be dealt with by over-the-counter insect control devices or products. If these fail to control infestation, Logan Extermination Services is contacted.

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F. QUALITY CONTROL

- FES personnel monitor the effects of pest control.

G. INTERPRETATION

If these procedures are followed correctly, insects and rodents will be well controlled at FES.

H. ASSOCIATED DOCUMENTS/REFERENCES

1. N/A

I. REVISION HISTORY

1. Original

J. APPENDICES

- N/A

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