Fish Health Laboratory Quality Assurance Program
Basic Requirements and Application for Tier II Recognition

(After reading this document, applicants should read the accompanying guiding document for specific additional instructions on timetables and logistics related to application.)

Introduction/overview

In recent years, the Fish Health Section of the American Fisheries Society launched an initiative relating to quality assurance for the operation, procedures and results produced from aquatic animal laboratories. The first phase of this process, titled Tier I – Prequalification has already been instituted at several laboratories throughout the U.S.

This application relates to the second level of this process entitled "Tier II – Recognition". There are several goals for this next level, including "raising the bar" even further from Tier I, verification that many of the accomplishments asserted in Tier I have been achieved, and finally, preparation for potential entry into a laboratory network or actual accreditation with an existing program. To accomplish these goals, laboratories will need to achieve an integrated quality management system (QMS).

Application procedures

A. Thoroughly review each category in 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 $1,200 non-refundable application fee. Make check payable to FHS/AFS.

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

D. The application will be reviewed and approved or denied within 6 months of completing and submitting all required materials.

Section #1: Tier I Compliance Records

Note: Section #1 - Compliance records are not included in this example to reduce document size

Required material: (should be included as part of the Quality Systems Manual, see Section 3)

Initials

AK a. Provide date and confirmation of Tier I Prequalification.

AK b. Provide an updated copy of your laboratory manual, including a description of any specific operational changes associated with standard procedures.

AK c. Provide two years of equipment calibration and standardization records (e.g. calibration invoices, freezer/incubator records, etc.) that have been collected since receiving Tier I approval.

AK d. Provide updated CV and qualifications of all employees.

Tier II (Recognition) Application for Fish Health Laboratories
American Fisheries Society/Fish Health Section

Section #2: Laboratory Information

Aquatic Animal Health Laboratory
Facility

Colorado Parks and Wildlife
Agency/Laboratory Affiliation

122 E Edison St
Laboratory Address

(970) 842-6308
Laboratory Phone Number

Brush, CO 80723
City, State, Zip Code

April Kraft	970-842-6304	april.kraft@state.co.us
Laboratory Director	Phone Number	Email
Victoria Vincent / John Drennon	970-842-6312	victoria.vincent@state.co.us
QA Manager	Phone Number	Email
		john.drennnon@state.co.us

Laboratory Biologists and Technicians:

April Kraft, laboratory director
John Drennan, fish pathologist
Laura Gerk, laboratory technician I
Ashley Malmlov, aquatic veterinarian
Victoria Vincent, laboratory technician II
Colby Wells, aquatic veterinarian
Carrie Brace, temporary laboratory technician
Katie Fletcher, temporary laboratory technician
Caroline Johnson, laboratory technican

Indicate which pathogens are routinely tested for during inspections or diagnostic cases which your lab wishes to recognized for expertise:

Viruses

- [x] IHNV Infectious Hematopoietic Necrosis Virus
- [x] IPN Infectious Pancreatic Necrosis Virus
- [x] VHS Viral Hemorrhagic Septicemia Virus
- [x] LMBV Largemouth Bass Virus
- [ ] CCV Channel Catfish Virus
- [ ] Other Viruses:

CTV cutthroat trout virus

Bacteria

- [x] *Renibacterium salmoninarum*
- [x] *Aeromonas salmonicida*
- [x] *Yersinia ruckeri*
- [x] *Flavobacterium psychrophilum*
- [x] *Flavobacterium columnaris*
- [x] *Aeromonas hydrophila*
- [ ] *Edwardsiella ictaluri*

Other bacteria:

Parasites

- [x] *Myxobolus cerebralis*
- [ ] *Schyzocotyle acheliognathii*
- [ ] *Ceratonova shasta*
- [ ] Other Parasites:

Section #3: Quality Manual

As part of an overall quality management system (QMS), the quality management system manual is an organized compilation of all the documents relating to quality assurance in the laboratory. The Quality Management System manual is the document that describes the planned and systematic activities used in the laboratory to ensure a level of quality will be achieved and maintained by the agency, lab, etc. This document should serve as the guideline for laboratory audits and for all questions related to quality management within the laboratory. The document is actually an outline of how the laboratory functions and operates. All of the work completed for the Tier 1 certification will be used in the QMS manual. The QMS manual will need a management section that most labs did not include in their Tier 1 application. There are two main sections to a quality management system: Management requirements and Technical requirements. **These requirements are listed in Appendix A.**

Specific information on creating this manual will be covered during the required QMS onsite training at Ames, IA, as mentioned later in this application.

Required material:

Initials

AK a. Provide a copy of the laboratory QMS manual.

Section #4: Biosafety Level 2 Verification

Tier I required laboratories to assert that they met all the requirements for Biosafety Level 2 (BSL-2). The Tier II application is requiring laboratories to be BSL-2 certified. Applicants should include a letter of approval or a letter detailing corrective actions and evidence for compliance from qualified individuals which could consist of a USDA/APHIS Veterinary Medical Officer, a State or university safety officer or other qualified individuals (with committee pre-approval). All correspondence should be included in the QMS manual. **More information regarding BSL-2 is included in Appendix B + C.**

Required Material:

Initials

AK a. Provide a BSL-2 compliance letter from a qualified inspector associated with entities such as USDA/APHIS, Veterinary Medical Officer, state or university safety officer or other qualified individuals that have been pre-approved by the QA/QC Committee.

Section #5: Training

Laboratory management must ensure the competence of all personnel performing the testing outlined in approved and current written protocols. Personnel shall be qualified to perform an assay on the basis of education, experience and or demonstrated skills. Training must be documented. Provide two years of the most recent training records for all laboratory personnel.

Specific training requirements on quality management systems for personnel in AFS-FHS Tier-II Laboratories includes the following:

i. The Laboratory Director and/or QA Manager must attend National Animal Health Laboratory Network's (NAHLN) multi-day onsite training on quality management systems at Ames, IA. Laboratories outside the U.S. may substitute a comparable course, with prior Committee approval.

ii. All other laboratory personnel must complete the self-directed online training course on laboratory quality management (https://apps.aoi.wsu.edu/qms/).

(1) Submit training records, which include a self-test for each module.

(2) The committee **highly recommends** the QA manager oversee this process to ensure training is done in a meaningful fashion over an adequate and reasonable period of time.

iii. Certificates of training will be examined by the committee and/or an internal auditor.

Required material: **Note: Section #5 - Employee online QA certificates and laboratory training records are not included in this example to reduce document size**

Initials

AK a. Provide confirmation (certificate of completion) that the Lab Director or QA Manager has attended the NAHLN QMS training in Ames, IA.

AK b. Provide all employee certificates associated with completion of the online QA training course.

AK c. Provide all personnel training records for laboratory competency for the past two years.

Section #6: Proficiency Testing (PT)

Tier II laboratories are expected to conduct and maintain proficiency testing. **Additional information and references for proficiency testing can be found in Appendix D.** Results of testing will be analyzed during the initial internal audit/GAP analysis. The following proficiency testing will be required:

i) The lab-defined audits will be conducted onsite by the Lab Director and Quality Assurance Manager, but the initial audit will also be coached and directed by NAHLN personnel and/or a QA Committee member.

ii) A record of schedule, details and results will be maintained in the QMS manual.

iii) Proficiency testing will begin with bacterial fish pathogens and include identification and sensitivity testing.

iv) The laboratory Director or QA Manager will provide lab personnel with blind samples for identification (should be one of the designated bacterial pathogens of laboratory's asserted expertise).

v) The Veterinary Laboratory Association (http://www.vetlabassoc.com/quality-assurance-program/) offers a comprehensive proficiency program for aquatic bacterial pathogens and histopathology. The QA Committee can assist if needed to help provide other sources of samples (USFWS, ATTC, other labs, etc.).

vi) Results of testing, at present, will remain confidential between the testing laboratory, the auditors and the QA Committee.

Note: The nature and scope of proficiency testing required in subsequent years will likely change as reagents, testing methods and professional services become available.

Required material:

Initials

AK a. Provide completed proficiency test results to Committee once the internal audit has been scheduled.

Section #7: Internal Audit/Gap Analysis

Specific quality control requirements for AFS-FHS Tier II Laboratories include an internal audit/GAP analysis. AFS-FHS Tier II Laboratories are expected to have lab-defined quality control procedures embedded as internal audits into their quality management system. These procedures are monitored to ensure the validity of test results and calibration of testing equipment.

i. The lab-defined audits will be conducted onsite by the Lab Director and Quality Assurance Manager, but the initial audit will also be coached and directed by NAHLN personnel and/or QA Committee member.

(1) Committee will arrange a date with NAHLN personnel.

(2) NAHLN personnel have limited time, so the number of audits/year is limited and would be ideally scheduled within a six month window of submitting the Tier II application.

(3) The committee will fund travel expenses for auditors.

(4) A vertical approach to gap analysis will be used. In a vertical approach gap analysis randomly selected cases are audited from when the samples arrive at the laboratory through reporting of the testing results. All documentation on each case will be reviewed. This may include safety procedures, paperwork or electronic records on accessioning, chain of custody on samples, testing records, review process of testing prior to reporting and reporting of test results, as well as all other Tier I requirements. Applicants are expected to produce a two year history of all records for review.

ii. A sample list is available at Appendix E to help prepare for the audit.

iii. Committee will review the report of Auditor/QA Manager and any GAP analysis recommendations and corrective action requirements before Tier II applications are approved.

Required material:

Initials

VM a. Schedule a time and date with the QA committee chairperson (Chris Wilson, 435-757-7493) to schedule and conduct an audit.

____ b. *(Committee use only) – Application and documentation of completed audit received.*

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	1Oct2020
	Date
Aquatic Health Manager	
Title	

AQUATIC ANIMAL HEALTH LABORATORY

QUALITY MANUAL

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2. Document Structure

Quality Manual External Documents

QA-001 – Organizational Chart and Staff Responsibilities
QA-002 – Code of Ethics & Conflict of Interest
QA-003 – Confidentiality and Pathogen Reporting
QA-004 – Corrective/Preventative Actions
QA-005 – Internal Audits
QA-006 – Safety and Health Manual
QA-007 – Subcontracting Services
QA-008 – Proficiency Testing
QA-009 – The Grandfather Clause
QA-010 – Master List of SOPs and Appendices
QA-011 – Pest Management and Control Plan

Technical Procedures

See SOP manuals for procedures, forms, equipment operation, and maintenance logs for each section.

SOP-AD	**Administration**
SOP-BACT	**Bacteriology**
SOP-VI	**Virology**
SOP-PCR	**Polymerase Chain Reaction**
SOP-PTD	**Pepsin Trypsin Digest**
SOP-AI	**Complete Inspections**
SOP-FD	**Field Diagnostics**

Forms

AD-Appendix A	**Administration Forms**
BACT-Appendix A	**Bacteriology Forms**
VI-Appendix A	**Virology Forms**
PCR-Appendix A	**Polymerase Chain Reaction Forms**
PTD-Appendix A	**Pepsin Trypsin Digest Forms**
AI-Appendix A	**Complete Inspection Forms**
FD-Appendix A	**Field Diagnostics Forms**

3. Quality Policy

i. The AAHL's Quality Management System (QMS) operates in accordance with the American Fisheries Society QA/QC program and consists of the Quality Manual, internal policies, procedures, and instructional materials that enable the laboratory to ensure the standardization and quality of its tests and diagnostic interpretations.

AAHL employees are familiar with the QMS and all associated documents, and are committed to a safe work environment, good professional practice, and technical competence. The AAHL complies with all State of Colorado regulations and internal policies to ensure the integrity of tests, reliability of accurate results, and the timeliness of results reporting, while adhering to the procedures and policies outlined in the QMS.

4. Management Requirements

a. Organization and Resources

i. The AAHL is funded by Colorado Parks and Wildlife revenue. The AAHL's mission statement is:

To protect, monitor, and enhance the health of aquatic wildlife and aquatic systems in the State of Colorado in accordance with Colorado state statutes.

The mission statement is accomplished by providing aquatic health inspection, diagnostic, research, and support services for state-managed hatcheries and waters.

ii. The Laboratory Director maintains an up to date organizational chart, QA001: Organizational Chart and Staff Responsibilities, which provides lines of authority and responsibilities for each position at the AAHL.

iii. Management ensures that employees conduct themselves in an ethical manner and are free of external pressures and conflicts of interest according to QA002: Colorado Employee Handbook.

iv. Management ensures that pathogen reporting is handled according to QA003: Confidentiality and Pathogen Reporting.

v. The Quality Assurance (QA) Coordinator and Safety Coordinator report directly to the Lab Director, who will function as the back-up QA and Safety Coordinator.

vi. The Laboratory Director and QA Coordinator have the responsibility of supervising and administering the Quality Management System (QMS) while providing the necessary resources and environment in which quality work can be safely produced.

b. Quality Management System

i. The QA Coordinator is responsible for scheduling biannual QA meetings and ensuring all significant information discussed at each QA meeting is documented and available to staff members upon request.

ii. The Laboratory Director is responsible for scheduling internal audits and the QA Coordinator is responsible for training and supervising the audit team members according to QA:005 Internal Audits.

iii. The Director and QA Coordinator are responsible for addressing and correcting nonconforming work and client complaints with the use of corrective/preventative actions where warranted.

iv. The QA Coordinator is responsible for training personnel on the Quality Management System, and management of all QMS related documents.

c. Document Control

i. Internal Policies

1. All AAHL employees may review and revise policies.

2. Only the Laboratory Director has the authority to review and authorize for use policy amendments or revisions to policy documents.

3. The review and authorization process must occur every other year.

ii. SOP Documents

1. All AAHL employees may write, review, or revise SOP documents.

2. All new and revised technical documents must be reviewed and approved by the Director, Safety Coordinator, and QA Coordinators.

3. Only the Director has the authority to authorize for use new or revised SOP documents.

4. The review and authorization process must occur every other year.

iii. The QA Coordinator is responsible for ensuring staff has access to current, controlled copies of all QMS documents as appropriate to their job responsibilities, as well as archiving retired documents along with any associated revision tracking forms.

iv. QMS documents are uniquely identifiable and cross-referenced. The document numbering format will adhere to the format presented in section 2 of this document.

d. Subcontracting of Tests

i. Any laboratory work subcontracted by the AAHL will follow the guidelines outlined in QA007: Subcontracting Services.

e. Purchasing Supplies and Services

i. Goods and services are purchased per CODNR regulations and guidelines, which can be found in The Financial Services Manual.

ii. Requests for purchase of goods intended for use in laboratory tests must be submitted to administration. The purchase of new or different reagents or products with the capability to impact quality of results are first approved with the Director or Fish Pathologist.

iii. On receipt, all items are visually inspected to ensure compliance with order specifications and quality criteria defined for the handling and storage of those reagents/materials that have the potential to affect the quality of laboratory work. The individual inspecting the items must date and initial the product and packing slip in upper right-hand corner.

f. Control of Nonconforming Events

i. The AAHL classifies any condition or technical error that resulted in a control failure, an incorrect result being reported, a delay in turn-around, a client complaint, or a safety violation as a nonconformance requiring immediate investigation and correction, to be recorded and filed with the QA Coordinators.

ii. The AAHL makes every effort to detect and promptly correct conditions that have or could adversely affect the quality of tests and test results including the use of corrective and preventative actions where warranted.

iii. All AAHL employees are responsible for maintaining the quality of work conducted by the laboratory, and have the authority to stop testing if the quality of work or safety is jeopardized. The Director has the authority to resume work once quality and safety conditions have been met.

g. Corrective and Preventative Actions

i. The Director and/or QA Coordinators has the responsibility to implement corrective actions, document any changes to operational procedures and schedule follow up audits to ensure the effectiveness of the corrective action according to QA004: Corrective and Preventative Actions.

ii. Corrective actions will be reviewed on a biannual basis at QA meetings, and areas for preventative actions identified.

h. Records

i. All paper record data-entry is legible, recorded in ink, and attributable to the individual by date and initials. Any data alterations require a single strike through with date and initials.

ii. The AAHL uses state regulated servers and hardware for storage and backup of electronic data. Access to this data requires the usage of a state designated individual employee login and password, as well as access to the Colorado Department of Natural Resource's secure network, or VPN access to that network.

iii. Case History Records

1. A case history associated record is defined as any document containing information pertaining to the collection, transport, processing, and results reporting of a case.

2. All case history associated records are clearly identified with the assigned case history number.

3. All original case history associated paper records are maintained for a minimum of 7 years.

iv. Financial (Purchasing, Billing) Records

1. All invoices, packing slips, and One Card documents must be organized and filed appropriately per ADMIN SOPs.

2. All original financial records are maintained on-site for a minimum of 2 years, at which point they are sent to DNR's billing department.

v. QMS (Internal Audit, Corrective and Preventative Actions, Training, Equipment Maintenance/Calibration, Laboratory Cleaning) Records

1. The QA Coordinator is responsible for ensuring QMS documents are current and available to staff, as well as archiving, organizing, and filing completed/retired documents.

2. All original QMS records are maintained for a minimum of 7 years.

i. Internal Audits

i. The Director and the QA Coordinator have the responsibility to manage internal audits according to QA005: Internal Audits. Any nonconformance detected during an internal audit will initiate the corrective action process.

5. Technical Requirements

a. General

i. The AAHL staff will review the tests performed annually to assess their appropriateness in the context of client use/need, current and future training and qualification of personnel, selection and calibration of equipment, material and reagent availability, projected client needs, and resource allocation.

b. Personnel

i. The AAHL maintains current position descriptions, training records, and proficiency records for all technical personnel performing assays on a routine basis.

ii. Proficiency records are updated every three years via internal proficiency tests consisting of well-characterized blind sample panels.

c. Accommodation and Environmental Conditions

i. The AAHL maintains a safe, clean work environment with appropriate lighting, temperature control, biosafety, and physical security according to QA006: Good Housekeeping and Environmental Conditions.

ii. All AAHL employees are responsible for monitoring environmental conditions that have the capacity to impact the quality of testing.

iii. All AAHL employees have the authority to stop testing if the quality of work or safety is jeopardized. Nonconformance to allowable ranges requires immediate remedial action by the Director, QA, or Safety Coordinator. The Director has the authority to resume work once quality and safety conditions have been met.

iv. Incompatible activities are separated by barrier walls physical distance where applicable.

v. The AAHL allows visitors, including equipment and facilities maintenance workers, with the requirement that the visiting individuals sign in and out on the visitor's log. Visitors may be required to wear appropriate laboratory personal protective equipment as needed.

d. Test Methods

i. General

1. The AAHL uses appropriate test methods and procedures for all diagnostic work based on relevance of the test method, result interpretation, acceptability by the scientific and regulatory communities, and resource feasibility.

2. The AAHL uses test methods and procedures defined in the AFS Fish Health Bluebook that are specifically approved for the presumptive and confirmatory identification of prohibited and regulated fish health pathogens per Colorado State regulations.

3. Tests used are to be reviewed upon changes to related policies or regulations, as warranted.

4. Tests performed are controlled using verified positive and negative reference standards. All internal controls used must be, at a minimum, verified by two individual assays, one of which must be either PCR or sequencing. All controls are clearly identifiable with source and expected results information.

5. The AAHL has SOPs for all testing procedures used in diagnostic work, for operation, maintenance, and calibration of equipment, as well as the collection, transport, and storage of test specimens.

6. The AAHL reviews and verifies current version of technical SOPs every other year. Employees working directly with revised SOPs must complete a revision review within 7 days of receipt.

ii. Selection of Methods

1. The rationale for using specified methods (see D.i.1) will be provided at the request of any client or regulatory agency.

2. Technical SOPs contain enough detailed information to allow a trained and competent technician to perform the test properly within pre-established control limits without reference to other information sources.

3. Testing SOPs include at a minimum: a description of the assay, required documents, materials, and equipment, acceptable tissue/biological material fit for testing, environmental condition requirements for the handling, processing, and storage of specimens, reference standards required and their acceptable limits, safety requirements, data to be recorded, and the method of data analysis, presentation, and interpretation.

iii. Validation of Test Methods

1. The AAHL uses validated tests and will provide, on request, test performance criteria (e.g. sensitivity, specificity, analytic range) as reported in validation documentation from international or national reference sources, commercial kit suppliers, and peer-reviewed journals.

iv. Control of Data

1. All test and validation data generated is maintained for a minimum of 7 years.

2. Computer software is considered validated on acceptance from a commercial or regulatory agency.

3. Each employee uses their own computer lot-in and secure password to access, update, or modify test files, inventory files, or other laboratory files to maintain an audit trial.

4. Computers and associated equipment are maintained in an environment that ensures proper functioning, integrity, and security of data.

5. AAHL employees are obligated to protect data collected on private lands, as well as research data, from distribution.

e. Equipment

1. The AAHL maintains necessary equipment and related test items required for the proper performance of tests and equipment calibration.

2. Equipment relevant to test accuracy and precision is calibrated as needed. Calibration performance is monitored and conducted as specified on calibration forms.

3. AAHL employees using equipment or performing calibrations for the first time must complete required training and receive competency approval prior to use. This training is to be conducted by a QA Coordinator assigned trainer and documented on relevant training documents.

4. The AAHL maintains and annually updates an equipment inventory list documenting the equipment's unique identification affixed to equipment, manufacturer's serial and model number, verification that the equipment meets performance specifications for the associated tests/procedures, location within the AAHL facility, and history of maintenance performed including a record of malfunctions, damage, or repairs.

5. Maintenance plans and schedules are written for all critical equipment used in testing. Failure to comply with maintenance schedules will result in a corrective action.

6. Malfunctioning equipment or equipment past-due for calibration is marked with a red warning label stating "do not use" until the equipment is repaired/calibrated and returned to service.

f. Measurement Traceability

i. The AAHL documents traceability for all equipment, reagent and media lot/batch information to certified reference standards, and/or participation in proficiency test and inter-laboratory comparisons with other AFS Tier certified fish health laboratories.

ii. Reference equipment/standards are handled, maintained, and stored to ensure proper performance and accuracy.

iii. All biologic reference materials are verified to perform within the expected range prior to use, and after 1 year of storage in the absence of a supplier-provided expiration date.

g. Specimens and Specimen Handling

i. The AAHL advises clients on the optimal methods for collecting, handling, and submitting specimens via outreach and web-based instruction. When a sub-optimal specimen is received, the information is recorded on the collection form, reported back to the submitting client, and any necessary steps for further collection of samples is initiated. Furthermore, the AAHL will provide counsel to the collector on how to optimize handling techniques to better ensure samples arrive within sample-receiving parameters set forth by AAHL.

ii. The AAHL reserves the right to refuse testing based on inappropriate sample submission, or the receipt of a sub-optimal specimen.

iii. Specimens are received, handled, protected, retained, and disposed of according to technical SOPs. All specimens are handled and stored in such a way as to preserve sample integrity for potential future testing.

iv. Each set of specimens submitted to the AAHL is entered into the Case History Log Book and assigned a case history number at the time of receipt. Each rack/container of specimens is uniquely identified with case history and lot number and that identification is used in any internal or external reporting of results.

h. Ensuring the Quality of Test Results

i. The AAHL monitors the validity of test results using: internal quality controls, follow up when significantly unexpected results are reported by AAHL employees or clients, and the participation in AFS Tier Certified laboratory comparisons and proficiency tests as available.

i. Reporting and Storage of Test Results

i. AAHL laboratory results are reported in an accurate, clear, unambiguous manner.

ii. Each individual test report includes:

1. The name of the laboratory performing assay(s)
2. Case history number
3. Date of receipt of specimens
4. Specimen source (including water code)
5. Identification of the type of specimen(s) received
6. Test name and method used
7. Date testing was completed
8. Test results and interpretations
9. Unique identification on each page of the report
10. Identification of the person(s) authorizing the test results and interpretations.

iii. Each Fish Health Certificate (required when reporting results of specimens included in annual/complete inspections) includes:

1. The name of the laboratory issuing the certificate
2. Case history number
3. Date of receipt of specimens
4. Date of collection of specimens
5. Specimen source (including location type, water code, and water supply)
6. Identification of the type of specimen(s) received
7. Name of laboratory performing assay(s)
8. Test results
9. Sample collector
10. Four year history of the location
11. Unique identification on each page of the report
12. Identification with qualifications of the person(s) authorizing the test results and interpretations.

iv. For each set of specimens assigned a case history number, a case accession form is generated. The case accession form includes:

1. The name of the laboratory
2. Case history number
3. Date of receipt of specimens

4. Specimen source (including water code)
5. Case type (annual, troubleshoot, research etc.)
6. Identification of the type of specimen(s) received
7. Test name and method to be used for each specimen received
8. Test results and interpretations
9. Unique identification on each page of the form
10. Identification of the person filing all case history related documents for storage upon completion of testing and reporting of results, and date filed.

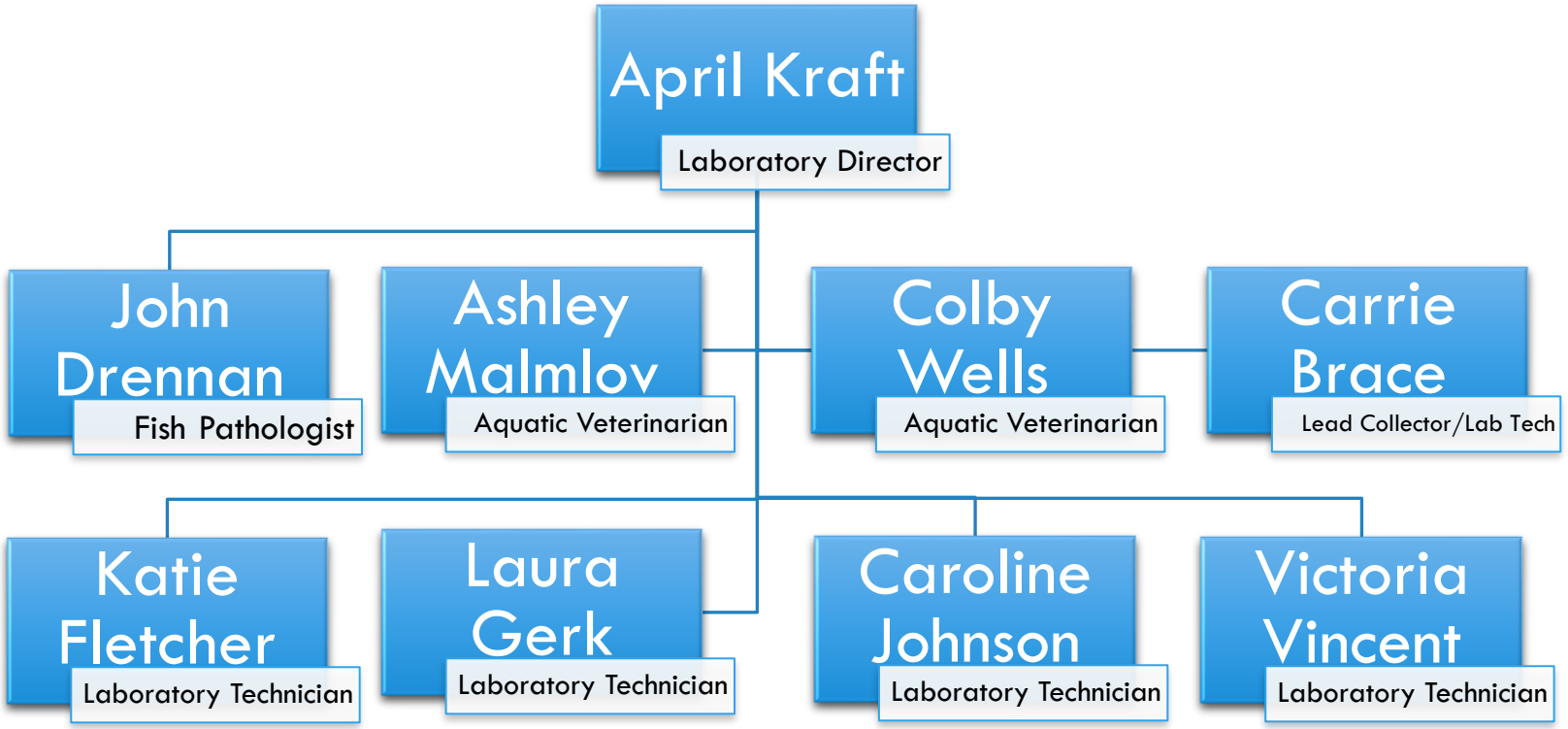
v. The case accession form and collection report must be filed, numerically, by case history number in the case progress folder. Test results are added as assays are completed. When all associated diagnostic assays are completed, the compiled documents associated with the case number are given to administration. Test results, definitive diagnosis (es), and/or classification of site (i.e. SPF, WD, BKD, etc.) are recorded in the remarks/diagnostic results section on the case accession form. All case associated documents are filed, numerically, by case history number in site-specific folders.

vi. All tests performed by subcontractors, including cases requiring the generation of Fish Health Certificates for private facilities, are identified with results reported, including any interpretation provided by the subcontractor.

vii. Related cases are cross-referenced when appropriate to the interpretation of the overall findings.

6. Revision History and Sources

a. BD Laboratory Quality Manual (2018).
b. 08/31/2020 – Implementation of Revision 01.
   i. Modified documents to reflect staff turnover and April Kraft as Laboratory Director.
   ii. Addition of QA010 Master List of SOPs and Appendices.
   iii. Addition of QA011 Pest Management and Control Plan.


April Kraft
Laboratory Director
John Drennan
Fish Pathologist
Ashley Malmlov
Aquatic Veterinarian
Colby Wells
Aquatic Veterinarian
Carrie Brace
Lead Collector/Lab Tech
Katie Fletcher
Laboratory Technician
Laura Gerk
Laboratory Technician
Caroline Johnson
Laboratory Technician
Victoria Vincent
Laboratory Technician

I. Annual Fish Health Inspections
- Inspection scheduling (AK)
- Analyzing hatchery inventory forms and requests (CB)
- Primary collector (CB)
- Fall collections: everyone chips in as available

II. Fish Health Troubleshoots
- CW, JDD, AM

III. Management
- Permanent staff (AK)
    i. Interstate communication
    ii. Hiring
    iii. Evaluations
    iv. Budget (AK, VV expenditure tracking)
    v. Exemptions (AK)
    vi. Importations (JDD)
    vii. Aquaculture permits (JDD)
    viii. Staff support
- Temporary staff (AK/VV)
    i. Hiring
    ii. Staff support
    iii. Training (LG,VV)

IV. Safety

- Ensuring required building safety codes are met and maintained (AK)

V. Administration

- T6 database management (AK, KF, VV, LG)
- Updating and maintaining AAHL webpage (AM)
- Placing orders for supplies, equipment, and services (AK, VV, LG)
- Vendor management (AK train:)
- CORE coding (AK train:)
- ONECARD billing (AK train:)
- Creating, distributing, and filing case history paperwork (LG, VV)
- Properly organizing and filing results of all assays performed from cases given an AAHL case history number (LG, VV)
- Fed case spreadsheet management (LG, VV)
- Creating, sending, and filling Fish Health Certificates (LG, VV)

VI. Lab duties

- Proper sample handling, completion of all required lab assays in a timely manner, and reporting of results (Lead lab tech per lab, other techs as available)
- Monitoring stock of supplies used weekly (Lead lab tech per lab, other techs as available)
- Equipment maintenance (Lead lab tech per lab/everyone who uses equipment)
- Temp logs (Temps, VV, LG)
- Cleaning (Lead lab tech per lab, all staff as available)
- Supplies ordering (AK, VV, LG)
- Cross training in all labs (All temps excluding admin, LG, VV)

- All staff as available for sample processing when needed

VII. QA/QC

- Implementation and adherence (Tier 1)
    i. QMS (All staff working in labs)
    ii. SOPs development and maintenance (VV and LG for routine lab work and administration, AM, JDD, and CW for Field diagnostics)
- Tier 2 implementation and adherence (all staff, focus for VV, AM)

VIII. Fish Health Board Meetings (AK, JDD, CW, AM)

IX. In state meetings

- Senior staff meeting (AK)
- Hatchery manager's meeting (2 people, more if day trip)
- Biologist's meeting (1 person, more if day trip)
- AFS meeting (1 person)
- CAA (1 person)

X. Out of state meetings

- DAWG (CW)
- ADAAP (CW)
- AA (Aquaculture America) (CW)
- AFWA (CW)
- WAFWA (AK)
- Rocky Plains (all pathologists)
- Western (3 people)

XI. Reporting drug use to other agencies (CW)

XII. INAD (CW)

XIII. Veterinary Feed Directives (CW, AM)

XIV. ANS inspections (VV, CB, CW as needed)

XV. Literature review for informing updates to and maintenance of (AM, AK, JDD, CW):

- Regulation
- Policy

XVI. Laboratory building maintenance (all staff as needed)

XVII. Fish health management duties (CW, JDD, AM)

XVIII. Trainings/Continuing education (AM, CW)

XIX. Education

- Hatchery/bio trainings (all permanent staff)

XX. Amphibian work (CW)

XXI. Lethal spawn collection and sample processing (all staff as available)

XXII. Research

- Processing samples for hatchery/bios (Lead lab tech and others as available)
- Deep sequencing implementation (AM)
- Diagnostic assay optimization (JDD, AM)
- Mentoring w/ graduate projects (AK, AM, JDD, CW)
- Helping grad students with sample assays in labs, supplies procurement (AK, VV, other techs as available)

XXIII. Biosecurity

- Audits (CW, AM)
- HACCP development (AM, CW, AK)

- FHA/HCP (AM, CW, AK)

XXIV. Grant research (for the AAHL) and application (AM)

AQUATIC ANIMAL HEALTH LABORATORY

QA002: Ethics and Code of Conduct

General Expectations

Code of Ethics & Conflicts of Interest

The holding of State employment is a public trust. State employees must carry out their duties for the benefit of the people of the State of Colorado. Article XXIX of the Colorado Constitution and Colorado Revised Statutes (C.R.S. 24-18-101) address ethics and conflicts of interest for State employees.

Independent Ethics Commission

The Independent Ethics Commission (IEC) is a constitutionally created independent commission and is charged with the implementation of Article XXIX of the Colorado Constitution. The purpose of the IEC is to give advice and guidance on ethics issues arising under Article XXIX of the Colorado Constitution and any other standards of conduct or reporting requirements as provided by law, and to hear complaints, issue findings, and assess penalties and sanctions where appropriate. The IEC has jurisdiction over all State executive and legislative branch elected officials and employees.

Accepting Gifts

The Colorado Constitution (art. XXIX, sec. 3) requires that local government officials do not accept gifts valued at more than $53 in any calendar year; rate adjusts every four years to account for inflation. Employees should never accept outside compensation (e.g., fees, gifts, rewards, etc.) for performance of state duties unless an opinion from the IEC approves such compensation. If an employee receives unapproved compensation and it cannot be returned, they should turn it over to their supervisor immediately. Direct all questions about Article XXIX to the IEC.

Political & Employee Activities

Employees may participate in political activities, subject to state and federal laws. However, no State facility or resource can be used for political activities and State employees are prohibited from using State time or the influence or authority of state employment to campaign for candidates. Employees have the right to join an employee organization, however, solicitation of members is not allowed during work hours without prior approval from their appointing authority.

Outside Employment

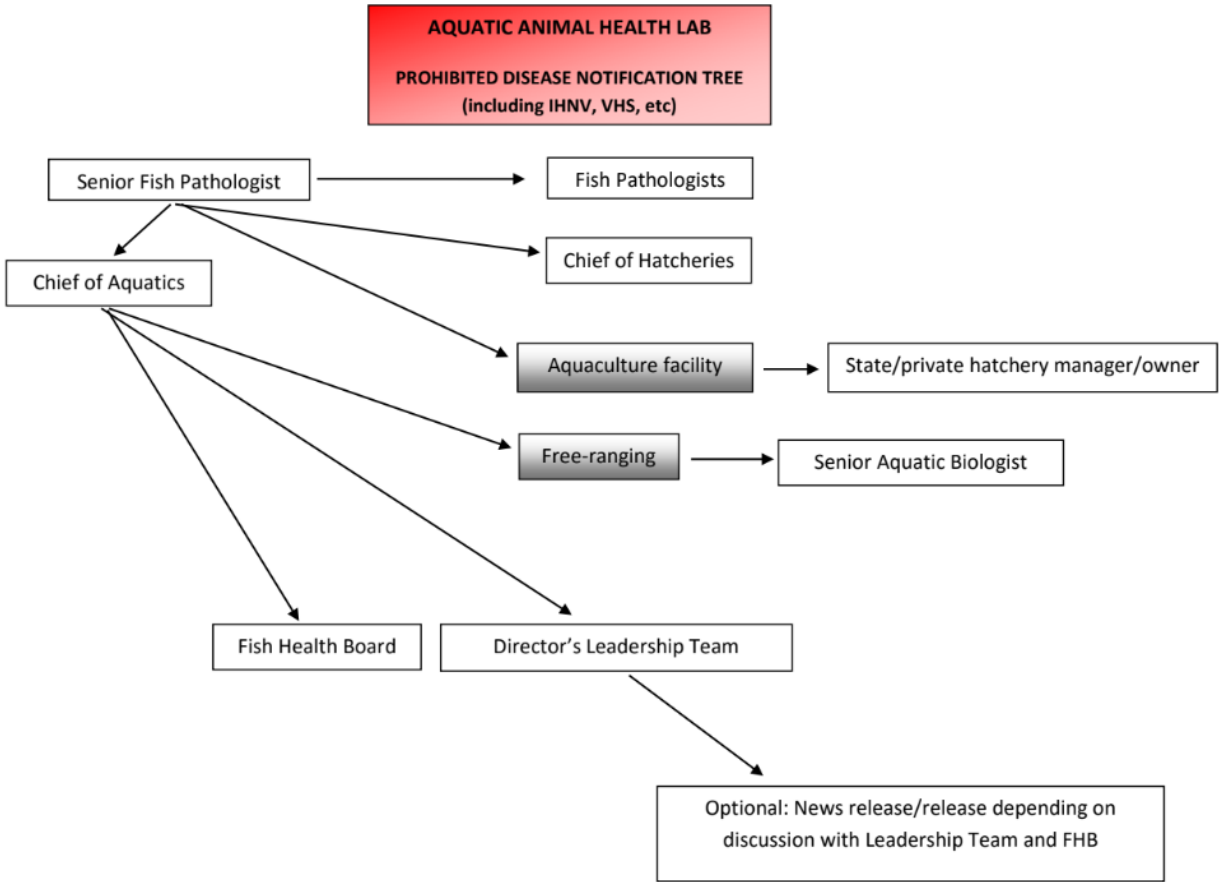
Employees must get advance, written approval from their appointing authority before engaging in outside employment. Outside employment with another employer or activity (e.g. business transaction, ownership etc.) that could be perceived as incompatible with the primary duties and responsibilities of an employee's State position is prohibited. Failure to obtain approval before beginning outside employment may result in corrective and/or disciplinary action.

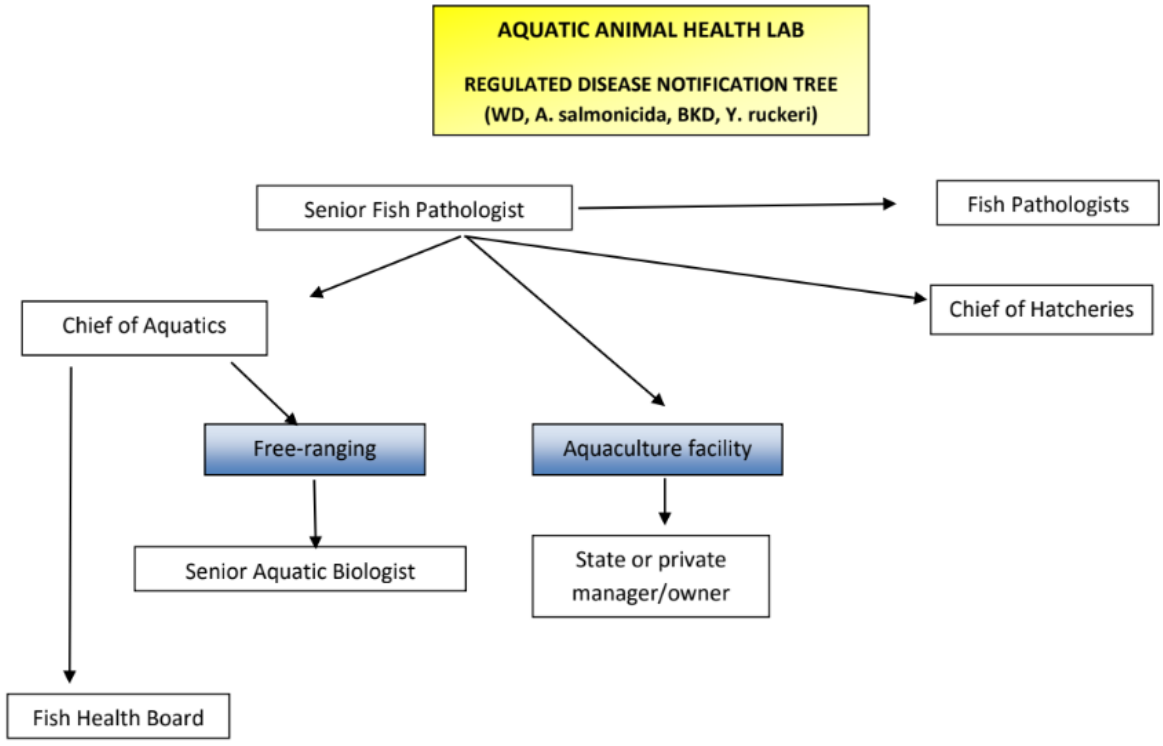
Independent Ethics Commission
www.colorado.gov/iec

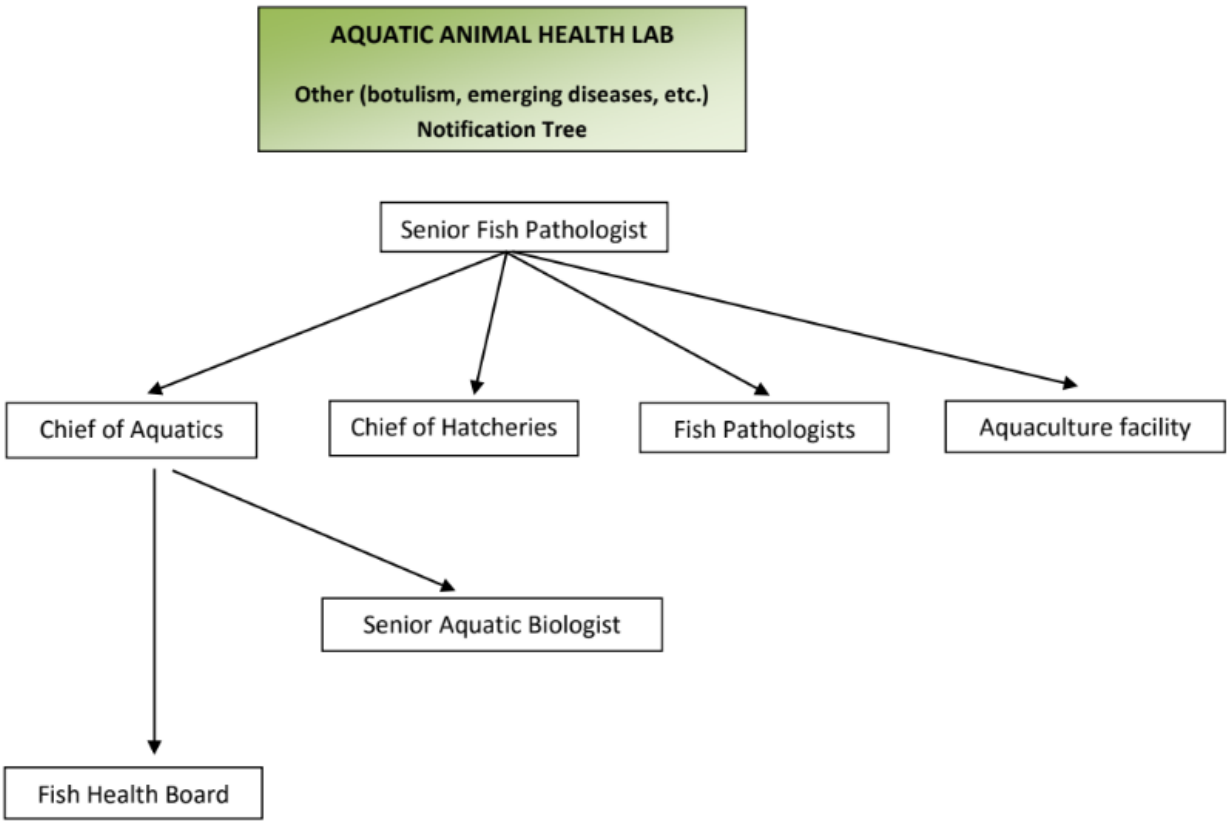
Independent Ethics Commission Handbook
www.colorado.gov/iec

AQUATIC ANIMAL HEALTH LABORATORY

QA003: Confidentiality and Pathogen Reporting


AQUATIC ANIMAL HEALTH LAB
PROHIBITED DISEASE NOTIFICATION TREE
(including IHNV, VHS, etc)
Senior Fish Pathologist
Fish Pathologists
Chief of Hatcheries
Chief of Aquatics
Aquaculture facility
State/private hatchery manager/owner
Free-ranging
Senior Aquatic Biologist
Fish Health Board
Director's Leadership Team
Optional: News release/release depending on discussion with Leadership Team and FHB


AQUATIC ANIMAL HEALTH LAB
REGULATED DISEASE NOTIFICATION TREE
(WD, A. salmonicida, BKD, Y. ruckeri)
Senior Fish Pathologist
Fish Pathologists
Chief of Hatcheries
Chief of Aquatics
Free-ranging
Aquaculture facility
Senior Aquatic Biologist
State or private manager/owner
Fish Health Board


AQUATIC ANIMAL HEALTH LAB
Other (botulism, emerging diseases, etc.)
Notification Tree
Senior Fish Pathologist
Chief of Aquatics
Chief of Hatcheries
Fish Pathologists
Aquaculture facility
Senior Aquatic Biologist
Fish Health Board

AQUATIC ANIMAL HEALTH LABORATORY

QA004: Corrective and Preventative Actions

1. Purpose & Scope

i. To describe the process for corrective and preventative actions used to ensure all work meets predefined expectations for quality and consistency set by the AAHL.

2. Definition and Terminology

i. Nonconformance – Any event during routine work at the AAHL that results in the nonfulfillment of a requirement.

ii. Corrective action – An action taken to correct a nonconformity, and prevent it from happening again.

iii. Preventative action – An action taken to prevent the occurrence of a nonconformity.

3. Corrective Actions

i. Upon the detection of a nonconformance, the Laboratory Director and/or Quality Coordinator(s) has the responsibility of initiating the corrective action process (AAHL-SOP-AD-8.01).

1. The nonconformance will be documented.

2. Responsibility for correcting the nonconformance will be assigned.

3. The immediate problem will be addressed with one or more of the following:
    a. Client contact
    b. Rework
    c. Reissuing of appropriate report

4. An investigation will be initiated within 10 days of report of nonconformance, which may include the use of documents such as:
    a. Maintenance logs
    b. Proficiency test results
    c. Training logs
    d. Test reports

5. The root cause of the nonconformance will be identified, and options for the corrective action will be recorded. The option that will reduce recurrence most will be chosen and implemented.

6. Any responsibilities or deadlines for the chosen plan will be communicated to appropriate personnel.

7. The plan will be completed and monitored as required to ensure success.

4. Preventative Actions

i. Upon discovery of the potential for a nonconformance to occur, the Laboratory Director and/or Quality Coordinator(s) has the responsibility of initiating the preventative action process (AAHL-SOP-AD-8.02).

1. Opportunities for improvement will be identified, e.g. areas of potential risk of nonconformance.

2. An Action plan will be developed, implemented, documented, and monitored for any change from the norm, including but not limited to:

    a. Financial/cost
    b. Time efficiency
    c. Ease of access/use

AQUATIC ANIMAL HEALTH LABORATORY

QA005: Internal and External Audits

1. Purpose & Scope

i. To describe the procedures for internal and external audits used by the AAHL.

2. Definition and Terminology

i. Conformance – An area or procedure found to comply with the QMS or SOP requirements.

ii. Deficiency – An area or procedure found to be in non-compliance with the QMS or SOP requirements.

3. Types of Audits

i. Gap analysis – A comparison of a procedure to QMS and SOP requirements; used to identify areas for improvement.

ii. Horizontal audit – Analysis of one activity/section of the QMS across multiple laboratories (e.g. document control, training documents, equipment logs etc.); commonly used to investigate adherence to corrective actions or changes in procedure.

iii. Sampling method– Selecting a random subset of activities to audit.

iv. Testing method – Observe a procedure and compare to the SOP.

v. Vertical audit – Analysis of all activities in a single process (e.g. an entire case from sample collection to reporting); used to examine a wide array of activities and their relationships to one another.

4. Internal Audit Procedure

i. The Laboratory Director is responsible for scheduling internal audits, which will be conducted by the QA Coordinator and/or Laboratory Director on an annual basis.

    1. It is the responsibility of the Laboratory Director to determine the necessity for more frequent internal audits, on the basis of the Quality Management System's integrity.

ii. The scope of an internal audit is dictated by the type of audit procedure chosen; either a horizontal audit of a minimum of two separate activities, or a vertical-gap analysis audit of a minimum of two cases, will be performed on an annual basis.

iii. SOP-AAHL-AD-7.01 Vertical Gap Analysis Audit, as well as the associated audit forms (AAHL-AD-APPENDIX-A.7) will be used to conduct internal audits.

    1. Deficiencies and conformances found during the audit will be recorded on the audit forms.

iv. It is the responsibility of all AAHL laboratory staff, QA Coordinator, and Laboratory Director to develop a plan and schedule to address any deficiencies found during an internal audit.

v. All internal audit paperwork, as well as any paperwork resulting from a review or corrective action as a consequence of said audit, will be filed with the QA Coordinator or Laboratory director.

5. External Audit Procedure

i. The Laboratory Director is responsible for scheduling external audits, which will be conducted by approved individuals/organizations as determined by the AFS FHS QA Program.

AQUATIC ANIMAL HEALTH LABORATORY

QA006: Safety and Health Manual

AQUATIC ANIMAL HEALTH LABORATORY

QA007: Send-Out Testing

1. Purpose & Scope

i. To describe the process for control of send-out testing to Referral laboratories used by the AAHL.

2. Definition and Terminology

i. Competent – Having the necessary ability, knowledge, or skills to do something successfully.

ii. Send-out testing – Any diagnostic testing the AAHL sends outside to a referral laboratory.

iii. Referral laboratory – An outside laboratory that provides diagnostic testing that the AAHL does not perform or in limited circumstances cannot perform in a timely manner. A referral laboratory can also be an outside laboratory that provides diagnostic testing results for private aquaculture facilities that the AAHL review in order to provide Fish Health Inspection Certificates or any other testing results required by State of Colorado law.

3. Procedure

i. A referral laboratory is a public or private laboratory that is approved by the Quality Control Manager.

ii. The AAHL reserves the right to send specimens to a referral laboratory to perform testing not done at the AAHL. This typically includes histopathology, toxicology, or other testing deemed appropriate by a State Fish Pathologist or Aquatic Veterinarian.

iii. When send-out testing results received from a referral laboratory that a private aquaculture facility utilizes for diagnostic testing, the AAHL will review the results to determine compliance for issuing any Fish Health

iv. Inspection Certificates or any other testing results required by State of Colorado law.

v. AAHL laboratory reports indicate tests performed at a referral laboratory by listing the test(s) and results and outside laboratory performing the work. The referral laboratory report is attached to the AAHL report.

AQUATIC ANIMAL HEALTH LABORATORY

QA008: Proficiency Testing

1. Purpose & Scope

i. To describe the process proficiency testing within the AAHL.

2. Definition and Terminology

i. Competent – Having the necessary ability, knowledge, or skills to do something successfully.

3. Procedure

i. Laboratory technicians will perform proficiency testing from one of the three following laboratories on an annual basis, in the following order:
   1. Bacteriology
   2. Pepsin Trypsin Digest
   3. Virology

ii. The Quality Coordinator(s) will provide blind samples to laboratory technicians for identification.

iii. These samples will consist of internal or approved external controls of the following pathogens:

   1. Bacteriology:
      a. *R. salmoninarum* (tissue sample for DFAT)
      b. *A. salmonicida* (cultured bacteria)
      c. *Y. ruckeri* (cultured bacteria)
      d. *F. psychrophilum* (cultured bacteria)
      e. *F. columnare* (cultured bacteria)

   2. Pepsin Trypsin Digest:
      a. 10 – 60 specimens from a known positive location (for PTD analysis)

3. Virology:
    a. IPNV (spiked tissue or frozen virus for cell culture)
    b. VHSV (spiked tissue or frozen virus for cell culture)
    c. LMBV (spiked tissue or frozen virus for cell culture)
    d. IHNV (spiked tissue or frozen virus for cell culture)
    e. CTV (spiked tissue or frozen virus for cell culture)

iv. Each laboratory technician will use and follow AAHL SOPs and protocols to process received specimens and identify pathogens present.

v. Each laboratory technician will present all results, paperwork, and prepared specimen reports to Quality Coordinator(s) for review and approval.

vi. The Quality Coordinator(s) will review and approve results.

vii. Any failure by a laboratory technician to identify pathogen(s) will result in a corrective action.

AQUATIC ANIMAL HEALTH LABORATORY

QA009: Employee Training Grandfather Clause

The effective implementation of the Quality Management System is October 17th, 2019. This date is also used for grandfathering of suppliers, employee training, and records.

Grandfathered employee training includes all tasks which the employee has been or is currently responsible for and which the employee has demonstrated proficiency/competency for an extended period.

Grandfathered Employee Training Record:

Employee Name: _____ Signature: _____ Date: _____

Supervisor Name: _____ Signature: _____ Date: _____

Employee Name: _____ Signature: _____ Date: _____

Supervisor Name: _____ Signature: _____ Date: _____

Employee Name: _____ Signature: _____ Date: _____

Supervisor Name: _____ Signature: _____ Date: _____

Employee Name: _____ Signature: _____ Date: _____

Supervisor Name: _____ Signature: _____ Date: _____

Employee Name: _____ Signature: _____ Date: _____

Supervisor Name: _____ Signature: _____ Date: _____

Employee Name: _____ Signature: _____ Date: _____

Supervisor Name: _____ Signature: _____ Date: _____

AQUATIC ANIMAL HEALTH LABORATORY

QA010: Master List of SOPs and Appendices

1. AD: Administration

- SOPs
  - AD 1.01 – Checking in Cases
  - AD 1.02 – Case History Spreadsheet and Lab Folders
  - AD 1.03 – Fish Health Inspection Certificates
  - AD 1.04 – Filing Fish Health Inspection Reports and Fed Case Log Sheet
  - AD 2.01 – Ordering Lab Supplies
  - AD 3.01 – OneCard Allocation
  - AD 4.01 – CORE Code Cover Pages
  - AD 4.02 – CORE Entry
  - AD 4.03 – CORE Approvals and Rejections
  - AD 5.01 – CARS Mileage Entry
  - AD 5.02 – Car Maintenance Scheduling
  - AD 6.01 – Proficiency Testing
  - AD 7.01 – Vertical Gap Analysis Audit
  - AD 8.01 – Corrective Actions
  - AD 8.21 – Preventative Actions
  - AD 9.01 – External Audits
  - AD 10.01 – SOP Training
- Appendices
  - AD App A – Administrative Forms
  - AD App B – Contact Lists

2. AI : Annual/Complete Inspections

- SOPs
  - AI 1.01 – Scheduling Collections
  - AI 2.01 – Cold Water Hatchery Collection
  - AI 2.02 – Cold Water Feral Collection
  - AI 2.03 – Warm Water Hatchery Collection
  - AI 2.04 – Warm Water Feral Collection
  - AI 2.05 – Shipping Samples
  - AI 3.01 – Mobile Laboratory
- Appendices
  - AI App A – Laboratory Forms
  - AI App B – MS222 MSDS

3. BACT: Bacteriology

- SOPs
  - BACT 1.01 – Laboratory Cleaning
  - BACT 1.02 – Bacti-Cinterator IV
  - BACT 1.03 – Thermolyne Heated Stir Plate
  - BACT 1.04 – Biochemical Safety Cabinet
  - BACT 1.05 – Wheaton Unispense Dispense
  - BACT 1.06 – Nikon Labophot-2
  - BACT 1.07 – Market Forget Sterilmatic Autoclave
  - BACT 1.08 – TR-403 Balance
  - BACT 1.09 – Cornwall Dispense
  - BACT 1.10 – Lab-Line Slide Warmer
  - BACT 1.11 – Incubators/Refrigerators/Freezers
  - BACT 2.01 – Tryptic Soy Agar
  - BACT 2.02 – Tryptone Yeast Extract Agar
  - BACT 2.03 – Blood Agar
  - BACT 2.04 – Cytophaga Agar
  - BACT 2.05 – Triple Sugar Iron Agar
  - BACT 2.06 – Oxidation Fermentation Media
  - BACT 2.07 – Simmon's Citrate Media
  - BACT 2.08 – Sulfide-Indole Motility Media
  - BACT 2.09 – Coomassie Brilliant Blue Media
  - BACT 2.10 – Shotts-Waltman Media
  - BACT 2.11 – Selective Kidney Disease Medium-2
  - BACT 2.12 – FA Buffer
  - BACT 3.01 – Gram Stain
  - BACT 3.02 – Hanging Drop Motility
  - BACT 3.03 – Cytochrome Oxidase Test
  - BACT 3.04 – Catalase Test
  - BACT 3.05 – 3% Potassium Hydroxide (KOH)
  - BACT 3.06 – API20E Test
  - BACT 3.07 – APIZYM Test
  - BACT 3.08 – *F. psychrophilum* DFAT
  - BACT 3.09 – *R. salmoninarum* Tissue DFAT
  - BACT 3.10 – *R. salmoninarum* Ovarian Fluid DFAT
  - BACT 3.11 – Complete Inspection Flow Chart
  - BACT 3.12 – Growth on TSA @ 22C

- BACT 3.13 – Growth on TYES @ 17C
- BACT 3.14 – Antibiotic Sensitivity Test
- BACT 3.15 – Endospore Stain
- BACT 3.16 – Acid Fast Stain
- BACT 4.01 – Ovarian Fluid for *R. salmoninarum* DFAT

- Appendices
  - BACT App A – Laboratory Forms
    - App A. 1: Collection Report Form
    - App A. 2: Case Tracking Form
    - App A. 3: Complete Inspection Isolate Assay Form
    - App A. 4: Troubleshoot Isolate Assay Form
    - App A. 5: Media Log
    - App A. 6: Equipment Temperature Logs
    - App A. 7: Autoclave Maintenance Log
    - App A. 8: Scale Calibration Log
    - App A. 9: Laboratory Cleaning Schedule
  - BACT App B – External Operation Manuals
    - App B. 1: Bacti-Cinterator IV
    - App B. 2: Thermolyne Heated Stir Plates
    - App B. 3: Biological Safety Cabinet
    - App B. 4: ThemoScientific Incubator
    - App B. 5: Wheaton Unispense Dispenser
    - App B. 6: Market Forge Autoclave
    - App B. 7: TR-Series Balance
    - App B.8: Nikon Labophot-2 Microscope
    - App B.9: Lab-Line Slide Warmer
    - App B.10: Maxi Mix Vortex
  - BACT App C – Flow Charts for the Presumptive Identification of Selected Bacteria
    - App C: Flow Charts for the Presumptive ID of Selected Bacteria
  - BACT App D – Plating Methods
    - App D: Plating Methods
  - BACT App E – Reagent List
    - App E: Reagent List
  - BACT App F – API Bacterial Profiles
    - App F.1: API20E Bacterial Profiles for *A. salmonicida* and *Y. ruckeri*
    - App F.2: APIZYM Bacterial Profiles

4. FD: Field Diagnostics (Troubleshoots)

- SOPs
  - FD 001 – Training Requirements

- FD 002 – Equipment
- FD 003 – Onsite Visit, History, and Signalment
- FD 004 – Observation of Fish
- FD 005 – Collection and Euthanasia
- FD 006 – Diagnostic Necropsy
- FD 007 – Troubleshoot Case Documentation
- FD 008 – Follow Up
- FD 009 – Treatment Recommendation Requirements
- FD 010 – Drug Treatment Guidelines
- FD 011 – Drug Prescription Requirements
- FD 012 – Treatment Effluent Requirements
- FD 013 – Sample Collection Report
- FD 014 – Troubleshoot Report
- FD 015 – Hatchery Drug Monthly Treatment Report
- FD 016 – Prescription Form
- FD 017 – Veterinary Feed Directive

- Appendices
  - FD App A – Field Diagnostics Forms

5. PCR: Polymerase Chain Reaction

- SOPs
  - PCR 1.01 – Laboratory Cleaning
  - PCR 1.02 – FisherSci Heat Block/Dry Bath
  - PCR 1.03 – Optimizer Workstation
  - PCR 1.04 – HandyStep Electronic Pipettor
  - PCR 1.05 – Scout Pro Balance
  - PCR 1.06 – Incubators/Freezers/Refrigerators
  - PCR 1.07 – FisherSci Marathon 16km Centrifuge
  - PCR 1.08 – Market Forget Sterilmatic Autoclave
  - PCR 1.09 – PTC-200 Thermal Cycler
  - PCR 1.10 – E-gel Power Base
  - PCR 1.11 – Hoefer HE33 Submarine Unit and Thermo E-C Apparatus
  - PCR 1.12 – UVP UV Transilluminator and Canon EOS T3 Camera
  - PCR 2.01 – Qiagen Kit and Reagents
  - PCR 2.02 – 1X TAE
  - PCR 2.03 – Primers
  - PCR 2.04 – Positive Controls

- PCR 3.01 – DNA Extraction
- PCR 3.02 – DNA Amplification
- PCR 3.03 – Gel Electrophoresis
- PCR 3.04 – Invitrogen E-gel Electrophoresis
- PCR 3.05 – DNA Quantification (Spectrophotometry)
- PCR 3.06 – Reporting Results

- Appendices
  - PCR App A – Laboratory Forms
    - PCR App A.1: Collection Report Form
    - PCR App A.2: Case Record
    - PCR App A.3: MasterMix
    - PCR App A4: MC Gel Report
    - PCR App A.5: RSAL Gel Report
    - PCR App A.6: Scale Calibration Log
    - PCR App A.7: Lab Cleaning Schedule
    - PCR App A.8 Autoclave Sterility Check
    - PCR App A. 9 Optimizer Workstation Log
  - PCR App B – External Operation Manuals
    - PCR App B.1: Dry Bath
    - PCR App B.2: Optimizer Workstation
    - PCR App B.3: HandyStep Electronic Pipettor
    - PCR App B.4: Scout Pro Balance
    - PCR App B.5: PrismR Centrifuge
    - PCR App B.6: PTC-200 Thermal Cycler
    - PCR App B.7: Sharp Carousel Microwave
    - PCR App B.8: Hoefer HE33 Submarine Unit
    - PCR App B.9: Thermo E-C Apparatus 250-90
    - PCR App B.10: UVP UV Transilluminator
    - PCR App B.11: Canon EOS T3 Camera
    - PCR App B.12: VWR Vortex
    - PCR App B.13: Argos Flexifuge
    - PCR App B.14: Biotek Epoch Plate Reader
    - PCR App B.15: Gen5 Program Software
    - PCR App B.16: Qiagen Kit Extraction Protocol
    - PCR App B.17: Qiagen Extraction Kit Handbook
    - PCR App B.18: DigiDocIt UVP
    - PCR App B.19: Docit Software
  - PCR App C – AFS Bluebook PCR
    - PCR App C.1: AFS PCR Protocols and Table 6.6
    - PCR App C.2: AFS BB *R. sal* Worksheet B
    - PCR App C.3: AFS BB *R. sal* Worksheet C

PCR App C.4: AFS BB *M. cerebralis* Modified PCR Procedures

- PCR App D – Reagent List
  PCR App D: Reagent List

6. PTD: Pepsin Trypsin Digest

- SOPs
  - PTD 1.01 – Laboratory Cleaning
  - PTD 1.02 – Reciprocal Shaking Bath
  - PTD 1.03 – Stir Plates
  - PTD 1.04 – Bio Gen Pro250 Homogenizer
  - PTD 1.05 – PR-7000 Centrifuge
  - PTD 1.06 – Olympus CH30 Biological Microscope
  - PTD 1.07 – Bio Gen Pro250 Homogenizer
  - PTD 1.08 – Scale
  - PTD 1.09 – Bleach Tubs
  - PTD 1.10 – Incubators/Refrigerators/Freezers
  - PTD 2.01 – Pepsin Preparation
  - PTD 2.02 – Pepsin Desiccant Cabinet
  - PTD 2.03 – Hydrochloric Acid Disposal
  - PTD 2.04 – Rinaldini's Solution
  - PTD 2.05 – Trypsin Preparation
  - PTD 2.06 – Albumin Preparation
  - PTD 2.07 – Dextrose/Sucrose Preparation
  - PTD 2.08 – Buffer Solution
  - PTD 3.01 – Checking in Samples
  - PTD 3.02 – Cooking Samples
  - PTD 3.03 – De-fleshing Samples
  - PTD 3.04 – Pepsin Digestion
  - PTD 3.05 – Trypsin Digestion
  - PTD 3.06 – Dextrose/Sucrose Filtration
  - PTD 3.07 – Reading Samples
  - PTD 3.08 – Preparing Samples for PCR Confirmation
  - PTD 3.09 – Spore Count Reporting
- Appendices
  - PTD App A – Laboratory Forms
    PTD App A.1: Collection Report Form
    PTD App A.2: Case Tracking Form
    PTD App A.3: Spore Count Analysis Form

PTD App A.4: Average Spore Count Report
PTD App A.5: Reagent and Media Logs
PTD App A.6: Label Templates (Rinaldini and sample)
PTD App A.7: Incubator Temp Logs
PTD App A.8: Autoclave Maintenance Log
PTD App A.9: Scale Calibration Log
PTD App A.10: Laboratory Cleaning Schedule

- PTD App B – External Operation Manuals
  PTD App B.1: Heated Stir Plates
  PTD App B.2: Biogen Homogenizer
  PTD App B.3: Precision Reciprocal Shaking Bath
  PTD App B.4: Market Forge Sterilmatic Autoclave
  PTD App B.5: PR7000M Centrifuge
  PTD App B.6: Frigidaire Refrigerator
  PTD App B.7: Microscopes
  PTD App B.8: Vortexes
  PTD App B.9 Scales
- PTD App C – AFS Bluebook Characteristics of *M. cerebralis*
  PTD App C: AFS Bluebook Characteristics of *M. cerebralis*
- PTD App D – Markiew, M.E., and Wolf, K. 1974
  PTD App D: Markiew, M.E., and Wolf, K. 1974
- PTD App E – Reagent List
  PTD App E: Reagent List

7. VI : Virology

- SOPs
  - VI 1.01 – Laboratory Cleaning
  - VI 1.02 – Laminar Flow Hood
  - VI 1.03 – Biological Safety Cabinet
  - VI 1.04 – Tissue Tearor
  - VI 1.05 – Incubators/Freezers/Refrigerators
  - VI 1.06 – Allegra X-30R Centrifuge
  - VI 1.07 – Rocking Platform
  - VI 1.08 – Market Forge Sterilmatic Autoclave
  - VI 1.09 – Laminar Flow Hood Filter
  - VI 1.10 – Manitowoc Ice Machine
  - VI 2.01 – MEM5 Plating and Overlay Media
  - VI 2.02 – MEM10 Growth Media
  - VI 2.03 – Dilution Media

- VI 2.04 – Incubation Media
- VI 2.05 – Transport Media
- VI 2.06 – Aliquoting Reagents
- VI 3.01 – Cell Culture
- VI 3.02 – Seeding Plates
- VI 3.03 – Tissue Sample Preparation
- VI 3.04 – Ovarian Fluid Sample Preparation
- VI 3.05 – Inoculating Plates
- VI 3.06 – Reading Plates
- VI 3.07 – Blind Passing/Passing Samples
- VI 3.08 – Resetting Samples
- VI 3.09 – Preparing Suspect CPE Samples for Shipment

- Appendices
  - VI App A – Laboratory Forms
    - VI App A.1: Sample Collection Form
    - VI App A.2: Case Tracking Form
    - VI App A.3: Record of Virus Assay
    - VI App A.4: Media Logs
    - VI App A.5: Equipment Temperature Logs
    - VI App A.6: Autoclave Sterility Check
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    - VI App A.8: Laboratory Cleaning Schedule
  - VI App B – External Operation Manuals
    - VI App B.1: Tissue Tearor
    - VI App B.2: Allegra X-30R Centrifuge
    - VI App B.3: Manitowoc Ice Machine
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    - VI App B.8: Olympus CK2 Inverted Microscope
    - VI App B.9: Laminar Flow Hood
    - VI App B.10: Biological Safety Cabinet
    - VI App B.11: Vortex
  - VI App C – Cell Monolayer and CPE Images
    - VI App C: AFS BB Identification of Viruses
  - VI App D – Media Calculations
    - VI App D: Media Calculations
  - VI App E – Media Recipes
    - VI App E: Media Recipes

- VI App F – Bluebook Suggested Seeding Ratios and Selection of Appropriate Cell Lines
  VI App F: AFS Bluebook Suggested Seeding Ratios and Selection of Cell Lines
- VI App G – Reagent List
  VI App G: Reagent List

AQUATIC ANIMAL HEALTH LABORATORY

QA011: Pest Management and Control Plan

1. Purpose & Scope

i. This guide is to aid in taking measures that prevent the ability of pests to gain access to the Aquatic Animal Health Laboratory (AAHL) building, and how to mitigate and eliminate their presence if they do access the AAHL.

1. Concerning pests: rodents, insects, and all pests from the outdoors that should not be in the building.

2. Guidelines for Management and Control of Pests in the Laboratory

i. Identification

1. AAHL staff must have knowledge of the appearance of the concerning pests and traces of said pests, making them easy to identify.

ii. Inspection

1. AAHL staff must be diligent about monitoring for pests and signs of pests in areas inside and outside of the building(s), as well as in shipments received by the AAHL.

iii. Sanitation

1. AAHL staff must be aware of and keep a level of general cleanliness within the building, and take steps to deprive pests of food sources.

    a. All areas within the building must be cleaned on a weekly basis.

    b. Any food must be sealed securely in containers and kept away from areas pests can access.

iv. Maintenance of Building

1. Maintain the building to avoid pest entry:

    a. Keep outside doors closed when not in use.

    b. Repair the building/doors/windows at the first sign of damage.

    c. Ensure window screens are undamaged, and create an intact barrier around the edges of the window frame.

v. Treatment

1. If pests do enter the building and intervention is required:

    a. If rodents/traces of rodents are found:

        i. Use gloves to dispose of traces and thoroughly disinfect area with 70% alcohol.

        ii. Set out d-CON bait stations in the area(s) in which they are identified.

        iii. Dispose of dead mice in a trash bag and place in in the outside dumpster.

    b. If insects/traces of insects are found:

        i. Spray with insect repellant and sweep up dead insects.

        ii. Dispose of dead insects in trash bag.

        iii. In extreme conditions, insect fogger can be utilized by strictly following the direction son the box.

Robert P. Ellis, PhD, CBSP, DACVM
University Biosafety Director
Professor, Department of Microbiology, Immunology and Pathology
D170 Research Innovation Center
Fort Collins, Colorado 80523-2025

Sara A. Cope, PhD
Assistant Biosafety Officer
D170 Research Innovation Center
Fort Collins, Colorado 80523-2025

Vicki Milano, Senior Fish Pathologist
Colorado Parks and Wildlife
Aquatic Animal Health Laboratory
122 E Edison
Brush, CO 80723

Dear Ms. Milano,
We visited Colorado Parks and Wildlife Aquatic Animal Health Laboratory June 24, 2019. Our visit was specifically to audit the Laboratory's compliance with the Biosafety Level 2 (BSL-2) and the AFS-FHS-QA standards and practices.

We documented that the Aquatic Animal Health Laboratory has implemented the BSL-2 standards and practices as listed on the Appendix C – Biosafety 2 Check List. As University Biosafety Officers, we are in a position to observe and audit a few hundred laboratories that are under BSL-2 containment protocols, both at our University and at other laboratory facilities. Aquatic Animal Health Laboratory practices are in line with the BSL-2 practices of other BSL-2 laboratories with which we are familiar. In reference to Item B8 on the checklist: "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." the Aquatic Animal Health Laboratory did inform us that any such incidents are reported and documented.

We had only minor suggestions that were made to the Director of the Laboratory and her staff. Over all, the Aquatic Animal Health Laboratory is very well managed and organized. The practices of the Laboratory definitely fulfill BSL-2 practices for the Aquatic Animal Health Laboratory.

If further information is requested, please contact Sara or me.

Sincerely,

Robert P. Ellis, PhD

Sara A. Cope, PhD

Robert Ellis, PhD, CBSP (ABSA), SM (ASM), DACVM, the university director of the biosafety office of the Vice President for Research at Colorado State University, along with his colleague Sara Cope, assistant biosafety officer, visited AAHL on June 24, 2019 for a biosafety inspection for biosafety level 2 approval. The biosafety inspection was structured according to the AFS Bluebook's Appendix C – Biosafety 2 Check list. The goal was to identify areas of improvement to acquire BSL-2 status. The following issues were identified; however, were considered minor enough by Dr. Ellis and Ms. Cope that it did not impede their approval of recommending AAHL for BSL-2 status.

Below are the concerns addressed by the AAHL from July 2019 – July 2020:

A.1: These policies are written in the Quality Manual under section 5.c.v.

A.2: Handwashing requirement signs are posted at the exit of every laboratory space.

A.3: These policies are written in the Safety Manual.

A.5: A needle-recapping policy has been added to our safety manual.

B.5: In the bacteriology lab there is a possibility of working with *Pseudomonas* spp., which is considered a BSL-2 pathogen. Safety information related to this is now listed on the Biohazard sign on the bacteriology lab door.

C.3: This section was not checked off as the team performing the inspection suggested that it might be appropriate to install acid spill kits in areas where we work with HCl. We have since installed an acid spill kit that is accessible in areas where we work with HCl.

United States
Department of Agriculture

Certificate of Training

This is to certify that

John Drennan

has satisfactorily completed

National Animal Health Laboratory Network
Quality Management System Training

Joey Kellum
Quality Manager
Mississippi State University

David Korcal
Quality Assurance Manager
Michigan State University

Pat Lukens
Quality Systems
Manager, WADDL

Kelly Burkhart
Microbiologist / QA
Specialist, NAHLN

Christina Loiacono
Associate Coordinator,
NAHLN

Date

August 6-10, 2018

Colorado Parks & Wildlife – Aquatic Animal Health Laboratory
Proficiency Testing Results Form

LABORATORY: Bacteriology
TEST: Pure bacterial culture identity and Antibiotic Sensitivity Testing

Employee:	April Kraft	Victoria Vincent	Laura Gerk
Date Started:	17SEP19	16SEP19	17SEP2019
Unknown specimen	*Aeromonas salmonicida*	*Yersinia Ruckeri*	*Yersinia Ruckeri*
Proficiency Assessment Tasks (√ = Check completed)			
Isolation of bacteria	√	√	√
Gram Stain	√	√	√
Triple Sugar Iron (TSI)	√	√	√
Cytochrome Oxidase	√	√	√
Motility	√	√	√
Hydrogen sulfide (H_2S)	√	√	√
Indole	√	√	√
API 20E	√	√	√
Antibiotic Sensitivity Test (AST)	√	√	√
Successful Identity of Specimen	√	√	√
Date Complete:	06 oct 19	02 oct 19	03 oct 19
Signature of Employee Completion			
Signature/Date of Quality Manager	16 oct 19 (John DRENNAN)		

Attach laboratory results of each employee's proficiency testing results

Tier 2 Gap Analysis-Internal Audit Summary

I would like to provide a summary of the Internal Audit that was conducted at Colorado Wildlife and Parks, Aquatic Animal Health Laboratory, Brush, Colorado on December 17, 2019. This was the final step that CO AAHL needed to complete for their Tier 2 Recognition as part of AFS Fish Health Section Fish Health Laboratory Quality Assurance and Quality Control Program. The audit or as Kelly Burkhart, National Animal Health Laboratory Network called it a "Gap Analysis" of the QA/QC program at CO AAHL. One thing I think the AFA FHC QA/QC committee needs to address is that this was actually an external audit or gap analysis as defined by the NAHLN. Internal audits are typically conducted by the QA/QC manager at each laboratory on a routine basis, but may be performed in a similar manner.

CO AAHL provided Kelly with all of their QA/QC documents prior to the Gap Analysis, including their Quality Management Manual and SOPs. Kelly acknowledge that she was reviewing those document prior to the Gap Analysis and provided the CO AAHL a brief summary of what she would be doing during the Gap Analysis, "I would like to begin with a short opening meeting, have a tour of your laboratory to understand sample flow, spend time reviewing your quality system documents and technical records, and conclude with a closing meeting when we will discuss observations identified".

I am not going to go in to any particular detail regarding the deficiency that Kelly identified during the Gap Analysis, but will summarize what I observed that may pertain to the USFWS Fish Health Centers as we develop Quality Management Programs at each FHC to meet the criteria for Tier 2 Recognition.

1. Review the requirements for Biosafety Level Two Laboratory requirements and address deficiency as needed prior to Tier 2 application and Audit-Gap Analysis.
2. The FHC will need to develop a Quality Management Manual for each lab. Trish Barbash and Kim True did modify the QA/QC document from Section 3 of the AFS FHS Blue Book that was part of the FHC Fish Health Policy. This document was submitted to headquarters by the FHC QA/QC committee in 2017 as part of the package that the FHC QA/QC committee wanted to include in the Tier 1 application. Headquarters felt that the document was not needed and therefore it was not submitted. It is the document that we will most likely work from as the FHC draft a Quality Management Manual. I will work to obtain other examples of QM manuals to use as templates. Many university veterinary diagnostic laboratories have theirs available on their websites and are excellent examples of QM manuals.
3. "Document Control" – Critical element of a laboratory's QA/QC program.
    a. Review and update SOPs – critical that it is documented how this process is done in your SOPs.
    b. Controlled Documents – "sticky notes" are not controlled documents. Lab procedure notes, lab bench procedure check off sheets if routinely used need to identified as "Controlled Documents". Procedure posters and pictures used for illustration of procedures or results need to be identified as "Controlled Documents". If you use a manufacture's procedure manual when doing a laboratory procedure that becomes a "Controlled Document".

    c. Controlled documents are identified in your SOPs or documented in the SOPs appendix and labeled when used in the laboratory as a "Controlled Document" and labeled as such.
4. Laboratory records for individual laboratories completed, dated, initialed and or signed by designated laboratory staff, reviewed by PL or QA/QC manager and signed. No "pencils" may be used to keep records!
5. Identify and document by SOPs how media logs, chemical inventories, equipment maintenance and calibration records are maintained for each laboratory.
6. Expired Media – there are exceptions to using expired media, but again those procedures need to be identified in your SOPs i.e., requalification and testing and how long is the requalification acceptable.
7. Staff training records need to be maintained and document. Needs and types of training should be identified. What qualifies a laboratory staff member to perform virology procedures?
8. Records Management – do our FHC SOPs adequately address how all of the laboratory records are managed, including hard copies and electronic data bases, etc.
9. Procedure validation – typically the procedures used have been documented and validated that we use for all of laboratory screening methods. If not you need to obtain that documentation and reference the method in a particular SOP. Records and SOPs are needed for media testing, cell line testing, etc.
10. Proficiency Testing – there are many options that I think the FHS QA/QC committee will find acceptable including internal and external. There are many ways that proficiency testing can be used to meet the QA/QC Tier 2 requirements, but we will need to establish our own procedures and needs for the FHC including developing specific SOPs to address proficiency testing.

These are basically the ten major areas that I felt that Kelly was interested in when she was conducting the Gap Analysis and conducting a specific vertical audit on a "Case History". Kelly noted that this is obviously a new and "young" process for the fish health laboratories in the QA/QC program. There are going to be deficiencies in the Gap Analysis – Audit. My observations and comments that I have heard from members on the FHS QA/QC committee is that the quality of each subsequent Tier 1 application improved and I believe that this will occur with the Tier 2 application process.

The final note I have is that the best document to work off of is Appendix E – Internal Audit Checklist in the AFS FHC Certification website tab under Tier 2 application procedures. This checklist was developed from the extensive audit process that the NAHLN uses for their laboratory audits. If you have a staff member that attended the NAHLN training in Ames, IA in 2018 they should be familiar with the checklist and the training material that was provided for Quality Laboratory Management. Visit with them if you have questions. Please download this checklist and become familiar with it and begin to address each item in the check list. Note particularly when they word "procedure" is used. You should have an SOP to address that procedure in the appropriate place in your SOPs.

I have recently mentioned that I would like to see the FHC QA/QC committee become active again to develop a general QM manual and work through the SOPs that we are deficient as identified from the audit checklist. The best approach I feel to review and develop action items from the checklist is to assign the QA/QC manger from each lab a section from the checklist similar how we assigned SOPs to be developed, written and shared for Tier 1 process.

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Sample Collection and Sample Collection Report

Date: 05MAR20

Auditor: Victoria Vincent

Sample Collection

SOP#(s)/Version Used: AI-2.01 Rev.#2 Implemented: 04SEP18

Training/Authorization Record for Sample Collection: None used at the time.

Additional Comments: There is still no training record for sample collection. -AK + VV would be grandfathered in.

Sample Collection Report - Sampling Location and Receiving Information

Case History Number (CHN): 19-091 Water Code: 04222

Date Collected: 28MAY19 Date Received: 28MAY19 AK

Name Location or Address/Owner: Poudre SFH / James Ingram

Is Sampling Location: ☒ Public ☒ Culture Facility ☐ Other :

☐ Private ☐ Free-ranging

Lab Destination(s): A = Aquatic Animal Health Lab

Case Type: ☐ CI ☐ SI ☒ RE ☐ TS ☐ EX ☐ Other: Collector(s): April Kraft, Victoria Vincent

Collector in Charge: April Kraft

Additional Comments: Numbers of samples written over older samples with no initials. Tawni Riepe not listed as a collector on the Report, but is on the accession form.

Sample Collection Report - Lot Information and Samples Collected

Does the Report Reflect the Following Information:

☒ AAHL Lot #s ☐ # Fish in Lot No ☒ Species ☒ Strain ☒ Age

☒ Lab Destination Listed for Each Sample Type Collected ☐ PTD Instrutions (if applicable) N/A

Additional Comments: Age hard to read. # fish in lot not required for this collection.

Equipment Temperature and Calibration Logs Reviewed: CI-001 (Equip. ID). Location listed is incorrect, Form updated Recently. 5/24/19-(3.8), 5/28/19-(3.7), 5/29/19-(4.5), 5/30/19-(3.9). No other equipment logs to review.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

NONE, however, training documents for sample collection should be generated ASAP- due @ the end of April~~*~~ August~~*~~.

4 27APR20W

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Sample Collection and Sample Collection Report

Page: 2 of 2
Date: 05MAR20
Auditor: VV

Further Audits Required to Complete CAP and Schedule/Deadlines: NONE, however, a Review in 6 months to assess the need/progress of sample collection training records must be done.

08SEP20:

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Case Log Form and Case Accession Sheet

Date: 30MAR20

Auditor: Victoria Vincent

Case Log Form

Case History Number (CHN): 19-091 Date Received: 05/28/19 AK

Source: Poudre River SFH Case Type: Research

Sample Type and Receiving Laboratory (List All):

(A) Bacteriology, (A) PCR

SOP#(s)/Version Used: AD-001, version 1. Last edited 2015

Training/Authorization Record: No training records currently.

Additional Comments: Training records and admin SOPs should be generated as quickly as possible/as need determined. There were none @ time of checkin, either.

Case Accession Sheet

Case History Number (CHN): 19-091 Date Received: 5/28/2019

Specimen Source: Poudre SFH

Water Code: 04222 Case Type: "RES"

Specimens Received (List Type and Number):

Bacteriology: BKD: #21

Parasitology: Ø

PCR: BKD: #21

ELISA: Ø

Other: Ø

Virology: Ø

Receiving Laboratory: A

Specimens Collected By: Kraft, Vincent, Riepe Date Collected: 5/28/2019

Method of Shipment: Hand carry Logged in By: VV

Comments: *OF for R. sal DFAT, kidney tissue for PCR.

Remarks/Diagnostic Results: DFAT (+), PCR (+)

Date Filed and Initials: 06/18/2019 VV SOP#(s)/Version Used: AD-005 V. #1 (2015)

Training/Authorization Record: No training records currently.

Additional Comments: Training records for admin should be generated ASAP/upon completion of SOP updates.

Vertical Audit of Diagnostic Accessions
Case Log Form and Case Accession Sheet

Page: 2 of 2
Date: 30MAR2020
Auditor: V. V.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

None- ~~a review in~~ training records for ADMIN SOPs must be generated upon completion of ADMIN SOP updates. The current projected time frame for SOP-ADMIN updates is the end of July.

Further Audits Required to Complete CAP and Schedule/Deadlines:

None- however, a review in 4 months to determine the completion of SOP-ADMIN updates, and the generation of training records for ADMIN SOPs is required.

01AUG20:

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Bacteriology DFAT Samples

Date: 30MAR20

Auditor: VV

Bacteriology Case Record - Case Information

Case History Number (CHN): 19-091 Date Received: 5/28/2019

Specimen Source: Poudre SFH Water Code: 04222

Number of Samples Received and Sample Type: Lot 2 BKD: #21

Additional Comments: 30MAR20 → Paperwork missing, filed in wrong folder.

Bacteriology Case Record - DFAT Record, DFAT Reading Record

Date Slides Prepared and Initials: 5/29/2019 BC* SOP#/Version Used: BACT-3.09 Rev. #3

Date Slides Stained and Initials: 03JUN19 BC* SOP#/Version Used: 11 Rev. #3

Date Slides Examined and Initials: →03JUN19 BC* SOP#/Version Used: 11 Rev. #3

Training/Authorization Records: Feb-Mar 2019; EJ + VV training

Proficiency Tested: Yes (No) If Yes, Date:

Number of Samples Prepared: 21

Pathogen of Interest: R. sal

Reagent Lot Number and Expiration Date Recorded:* 10311945 * EXP: not required

CHN for Positive Control Used: * Not Required @ the time assay completed

Additional Comments: * Initials not required on paperwork @ the time → got initials from BC's lab notebook.

Equipment Temperature and Calibration Logs Reviewed: BI-002 (17°C incb.), BI-004.1 (bact media fridge), BI-005 (Reagent fridge)*; * 8/14/18-8/19/19 missing. → found - just wasn't filed yet!

Bacteriology Case Record, DFAT Reading Record, Troubleshoot Testing Record, Complete Inspection Testing Record

Date Case Closed and Initials: 12JUN19 BC Reporting SOP#/Version Used: BACT 3.09 - Rev. 3

*Training/Authorization Record: Feb-March 2019; EJ + VV training.

Does Report Accurately Reflect the Following Information:

☒ # of Samples Tested (21) ☒ Test Performed (BKD) ☐ Results for Each Sample *

☒ Name of Report ☒ Pagination/End ☒ Dates Received/Tested (5/28/2019 - 03JUN19)

Additional Comments:

* Results for each sample found in BC Laboratory notebook. Since this case was finalized new paperwork to show results for each sample has been implemented:

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Bacteriology DFAT Samples

Date: 14APR20

Auditor: VV

BACT - Appendix - A.10: DFAT Reading Record.

Lab cleaning logs: 2019 BACT → fully filled out

Media Logs: SKDM 09APR19BC, 10APR20BC, 08MAY20BC.
No controls used as we do not have R.sal controls.

QMS Logs: EJ: all 9 VV: all 9
BC: all 9 AK: all 9

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit: None - temporary personnel should not be training other temporary personnel during their first season at the lab. A review to determine the status of the missing BI-005 temp log page should be done in 4 months 01AUG19: R.sal culture control?

Further Audits Required to Complete CAP and Schedule/Deadlines:

Reviewed by Laboratory Director (date and signature):

Reviewed by QA Coordinator (date and signature):

Filed (date and initial):

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Polymerase Chain Reaction Samples

Date: 30MAR20

Auditor: VV

PCR Case Record - Case Information

Case History Number (CHN): 19-091

Date Received: 5/28/2019

Specimen Source: Poudre SFH

Water Code: 04222

Case Type: "Subtype" Research

Target Pathogen: R. salmoninarum

☒ Traditional PCR ☐ qPCR

Number of Samples Received and Sample Type:

Additional Comments: 30MAR20 - Paperwork missing; filed in wrong folder.

PCR Case Record - Sample Testing and Results

Number of Samples Prepared: Lot 1: #21

Extraction Date & Initials: 5/28-5/29 AK

SOP#/Version Used: PCR-3.01 Rev. 1

Amplification Date & Initials: 5/29/19 AK

SOP#/Version Used: PCR-3.02 Rev. 1

Gel/Plate Date & Initials: 5/29/19 AK

SOP#/Version Used: PCR-3.03 Rev. 1

Spec DNA Date & Initials: Not filled out

SOP#/Version Used: None @ the time.

Extraction & Amplification (+) Control CHN: Not filled out, - not in SOPs from this time.

Training/Authorization Records: AK grand-fathered in to PCR.

Proficiency Tested: Yes (No)

If Yes, Date:

Date Case Closed and Initials: 5/29/19 AK

Reporting SOP#/Version Used: None @ the time.

Training/Authorization Record: AK grandfathered in.

Does Report Accurately Reflect the Following Information:

☒ # of Samples Received ☒ Test Performed ☐ Results for Each Sample*

☒ Name of Report ☒ Pagination/End ☒ Dates Tested

Equipment Temperature and Calibration Logs Reviewed: PCR-004 (AMP), PCR-005 (AMP freezer), PCR-015 (gel fridge), PCR-006 (EXT)

*Since this case was finalized, it was decided to require PCR reports for every gel ran - which will show ea. sample result.

Additional Comments:

We are getting more consistent at speaing DNA and filling that section of the report out. We are also very consistent at filling out the controls used.

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Polymerase Chain Reaction Samples

Date: 14APR20

Auditor: W

Lab cleaning logs: 2019 BACT → ✓
2019 PCR → Only filled out Aug → Nov. Empty: Jan–July + December.

Media Logs: ~~BACT~~: N/A

QMS Logs: AK: ✓ all 9 VV: ✓ all 9
BC: ✓ all 9

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit: Ensure cleaning checklists are being filled out PROPERLY.
REVIEW DIAUG19:

Make larger

Further Audits Required to Complete CAP and Schedule/Deadlines: None – have a Reminder meeting about filling out our case tracking forms + sperm DNA + ensuring PCR REPORTS are COMPLETED for every gel RAN.
DIAUG19:

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Sample Collection and Sample Collection Report

Date: 27APR20

Auditor: Victoria Vincent

Sample Collection

SOP#(s)/Version Used: AI-2.01 CWD-Hat-TissueColl. Rev #2. Implemented 04Sep18

Training/Authorization Record for Sample Collection: None used @ this time.

Additional Comments: Collected by Evan Jones

Sample Collection Report - Sampling Location and Receiving Information

Case History Number (CHN): 18-224 Water Code: 02356

Date Collected: 11·19·18 Date Received: 11·19·18

Name Location or Address/Owner: Roaring Judy Iso #2, Seth Firestone

Is Sampling Location: ☒ Public ☒ Culture Facility ☐ Other :
☐ Private ☐ Free-ranging

Lab Destination(s): A (KIS), (KID), (TSA), (PTD (HDS))

Case Type: ☒ CI ☐ SI ☐ RE ☐ TS ☐ EX ☐ Other: Collector(s): Evan Jones

Collector in Charge: Evan Jones

Additional Comments:

Sample Collection Report - Lot Information and Samples Collected

Does the Report Reflect the Following Information:

☒ AAHL Lot #s ☒ # Fish in Lot ☒ Species ☒ Strain ☒ Age

☒ Lab Destination Listed for Each Sample Type Collected ☒ PTD Instrutions (if applicable)

Additional Comments: 12 KIS, 40 KID, 40 TSA, 40 HDS

Equipment Temperature and Calibration Logs Reviewed: CT-001 (Equip. ID).
11/15/18 - (4) EJ, 11/19/18 (4) LG, 11/20/18 (4) LG, 11/21/18 (5) LG → 11/26/18 (4) LG. This gap in Recording due to holiday.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

None, however training documents for sample collection should be generated due by the end of August.

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Sample Collection and Sample Collection Report

Date: 27APR20

Auditor: VV

Further Audits Required to Complete CAP and Schedule/Deadlines:

Review of training documents for 1st sample collection.

1st August 2020.

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Case Log Form and Case Accession Sheet

Date: 27Apr20

Auditor: Victoria Vincent

Case Log Form

Case History Number (CHN): 18-224 Date Received: 11/19/18 AK

Source: Roaring Judy ISO #2 Case Type: Annual COMPLETE

Sample Type and Receiving Laboratory (List All):

Virology (A), bacteriology (A), M. cerebralis (PTD)(A)

SOP#(s)/Version Used: AD-001, version 1. Last edited 2015

Training/Authorization Record: No training Records implemented.

Additional Comments: Form Recently updated. Results section now called "comments." Date completed column Removed as it was never used.

Case Accession Sheet

Case History Number (CHN): 18-224 Date Received: 11.19.2018

Specimen Source: Roaring Judy SFH, ISO 2

Water Code: 023516 Case Type: CI

Specimens Received (List Type and Number):

Bacteriology: BKD: 60, TSA: 60

Parasitology: WD: 60

PCR: Ø

ELISA: Ø

Other: Ø

Virology: 60

Receiving Laboratory: A

Specimens Collected By: Evan Jones Date Collected: 11/19/2018

Method of Shipment: Hand Carry Logged in By: PS

Comments:

Remarks/Diagnostic Results: Empty.

Date Filed and Initials: 1/2/19 PS SOP#(s)/Version Used: AD-005 V.#1 (2015)

Training/Authorization Record: None currently implemented.

Additional Comments: Accession form could change "WD" to "PTD" to match collection form and case log form.

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Case Log Form and Case Accession Sheet

Page: 2 of 2
Date: 27APR20
Auditor: VV

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

Further Audits Required to Complete CAP and Schedule/Deadlines:
Review Progress of Admin training records.
August 2020.

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Virology: Tissue and OF Samples

Page: 1 of 1
Date: 27Aug20
Auditor: Victoria Vincent

Virology Case Record - Case Information

Case History Number (CHN): 18-224 Date Received: 11/19/2018
Specimen Source: Roaring Judy SFH IS02 Water Code: 02356
Number of Samples Received and Sample Type: Lot 1, KLS, 12
Additional Comments: Case accession says 120 samples → form has recently been updated to reflect # of vials received.

Virology Case Record - Record of Virus Assay - Form filed separately.

Sample Processing Date and Initials: 11/20/18 LG SOP#/Version Used: VI-3.03 Rev.#3 12/3/18
Date Samples Inoculated and Initials: 11/21/18 LG SOP#/Version Used: VI-3.05 Rev#3 12/3/18
Training/Authorization Records: June of 2018 by W. **
Proficiency Tested: Yes (No) If Yes, Date:
Number of Samples Processed: 12
Reagent Lot Numbers Recorded (MEM5, DIL, INC): 170CT18LG/16AUG18LG/06AUG18LG
Cell Lines Used and Pass Number: CHSE: 298 (3) LG / EPC: 145 (3) LG
Incubation Temperature(s): 15°C
Blind Pass Date(s) and Initials: 04DEC18 → Initials not req. on paper@time.
Does Report Accurately Reflect the Following Information:

☒ Dates Plates Read and Initials (Int. not req. @ time) ☒ Confluence of Cells ☒ Resets/Blind Passes
☒ Presence/Absence of CPE ☒ Presence/Absence of and Type of Contamination*

Additional Comments: 170CT18LG MEM5 missing *, there is a date for MEM5 of 16AUG18LG. → 13NOV18LG. 03OCT18 crossed out → no reagent info. Some Reagents expired → usual/Regular.
Media Logs Reviewed:
*Contamination/toxicity notes not always consistent from person to person.
Equipment Temperature and Calibration Logs Reviewed:
VI-004 (4°C): 11/20, 11/21: 4.1. Missing 11/23-25 (weekend/holiday)
VI-008 (4°C): 11/20 4.7 (LG), 21: 5.4 (LG), 26: 4.8 (LG), cleaned + defrosted 12/20 LG
VI-012.1 (Reagent Fridge): same dates. Steady 3-4 (LG), high of 4.3 in Dec.

Virology Case Record - Record of Virology Assay for Regulated Salmonid Inspections

Date Case Closed and Initials: 12/18/18 LG Reporting SOP#/Version Used: None made! ***
Training/Authorization Record: None - but the paperwork is filled out properly.
Does Report Accurately Reflect the Following Information:

☒ # of Samples Tested ☒ Test Performed ☐ Results for Each Sample → No but WE don't do this...
☒ Name of Report ☒ Pagination/End ☒ Dates Received/Tested

Additional Comments:

Equipment temp logs continued: VI-013 Culture Reagents + Media Same dates as others: From 11/20 - 12/20 13 days were recorded. High of 6; low of 2.
VI-004 Processing Room media + Reagents. Same dates, high of 6, low of 2
VI-005 Reagents Freezer Same dates - high of -18 low of -24

No calibration or autoclave logs used at this time.
Cleaning Records: 2018 virology: Nov 2nd all but mop, 9th same, 16th same, 23rd same, 30th same.
** Training conducted prior to SOP edits. Need to get an SOP Review Sign-Off Sheet made + add to SOPs.
*** VI-6304 Needs to include how to report Results as negative or not → how to fill out paperwork.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

Further Audits Required to Complete CAP and Schedule/Deadlines: Review to ensure "SOP edits sign-off" sheet is created + implemented: 01 Aug 20: VI-03.04 updated to include Reporting Results - complete paperwork.

Reviewed by Laboratory Director (date and signature):

Reviewed by QA Coordinator (date and signature):

Filed (date and initial):

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Bacteriology Culture Samples

Date: 30APRIL20

Auditor: Victoria Vincent

Bacteriology Case Record - Case Information

Case History Number (CHN): 18-224 Date Received: 11/19/2018

Specimen Source: Roaring Judy SFH ISO 2 Water Code: 02356

Number of Samples Received and Sample Type: LOT 1: TSA (40), BKD (40)

Additional Comments:

Complete Inspection Isolate Assay Form

Number of Samples Examined: None found → leads me to

Work Performed, Date, and Initials: believe that there was no growth on the TSA. It would be helpful if that was noted on the case Record sheet.

SOP#s/Version Used: BACT-# 3.12 Growth on TSA Rev.#3 (08/15/2020)

Training/Authorization Records: 8/22/18 VV → 10/16/18 VV. Ind. column not completed.

Proficiency Tested: (Yes) No If Yes, Date: Not Recorded *

Media Logs Reviewed: We don't Record the TSA that we use per case. ** ~~Didn't start Regularly Recording~~ ✓✓ TSA used up to 05DEC18 expired 05/31/2018 – all controls worked. ✓ Re-assay success!

Equipment Temperature and Calibration Logs Reviewed: 11/20-12/03: 7 days recorded. BI-001 (22°) Low of 22.2 high 22.6. BI-004.1 (media) high: 4.4 Low: 4.5. BI-005.1 (Reagents) high of 7.3 low: 6.5. As high as 9.5 prior → no range on sheet.

Additional Comments:

Bacteriology Case Record, (Complete Inspection Isolate Assay Form) = (N/A)

Date Case Closed and Initials: 12/3/18 EJ

Reporting SOP#/Version Used: BACT-3.12 (above) Not Reporting @ the time but since updated.

Training/Authorization Record: (above)

Does Report Accurately Reflect the Following Information:

- ☒ # of Samples Tested
- ☒ Test Performed
- ☐ Results for Each Sample ← Not done w/ TSA BKD sheets now available
- ☒ Name of Report
- ☒ Pagination/End
- ☑ Dates Received/Tested ← Not for TSA!

Additional Comments:

Need to adjust SOPs to require a note when CIAF is not used. ***

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Bacteriology Culture Samples

Page: 2 of 2

Date: 04/30/20

Auditor: W

Cleaning Records: 2018 BACT: 2nd NOV - all, 9th all but sweep, 16th all, 23rd none, 30th all. NO mopping - cleaning crew was mopping labs at this time.

No autoclave or scale calibration logs used at this time.

*As part of training process individuals are given a proficiency test. This was not recorded as that wasn't/isn't part of our training protocols.
**Decide to add BA lot to paperwork
***Add step to SOPs to require a note to be made when there's no growth on TSA.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

Further Audits Required to Complete CAP and Schedule/Deadlines:

Review to ensure * are met:
01AUG20:

Reviewed by Laboratory Director (date and signature):

Reviewed by QA Coordinator (date and signature):

Filed (date and initial):

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Bacteriology DFAT Samples

Date: 04/30/20

Auditor: Victoria Vincent

Bacteriology Case Record - Case Information

Case History Number (CHN): 18-224 Date Received: _____

Specimen Source: _____ Water Code: _____

Number of Samples Received and Sample Type: _____

Additional Comments: _____

Bacteriology Case Record - DFAT Record (DFAT Reading Record) - LN/A not in use @ time)

Date Slides Prepared and Initials: 11.19.18 EJ SOP#/Version Used: 3.09 Rev.3 08/15/18

Date Slides Stained and Initials: 11.28.18 EJ SOP#/Version Used: 11

Date Slides Examined and Initials: 11.28.18 EJ SOP#/Version Used: 11

Training/Authorization Records: 8/22 - 8/28 VV/KF - Blank sections & paperwork.

Proficiency Tested: Yes (No) If Yes, Date: _____

Number of Samples Prepared: 100 (# examined)

Pathogen of Interest: "R. sal"

Reagent Lot Number and Expiration Date Recorded: 1031945 → Exp. not required *

CHN for Positive Control Used: 15-202 → Not even req. @ the time. Kudos EJ

Additional Comments: Add sections to include exp. date & prepared date to media logs as well at case record.)
Expiration date not on reagent² so will not include on paperwork.

Equipment Temperature and Calibration Logs Reviewed: Same as TSA!

Bacteriology Case Record, DFAT Reading Record, Troubleshoot Testing Record, Complete Inspection Testing Record

Date Case Closed and Initials: 12/03/18 EJ Reporting SOP#/Version Used: Same as TSA

Training/Authorization Record: 11

Does Report Accurately Reflect the Following Information:

☐ # of Samples Tested ☐ Test Performed ☐ Results for Each Sample } same as TSA

☐ Name of Report ☐ Pagination/End ☐ Dates Received/Tested

Additional Comments: _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Bacteriology DFAT Samples

Page: _____ of _____

Date: _____

Auditor: _____

Cleaning records same as TSA

* Include expiration & Prepdate on R.sal conjugate!

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit: _____

Further Audits Required to Complete CAP and Schedule/Deadlines: _____

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Pepsin Trypsin Digest Samples

Date: 4/30/20

Auditor: V V

PTD Case Record - Case Information

Case History Number (CHN): 18-224

Date Received: 11/19/2018

Specimen Source: Roaring Judy SFH, ISO2

Water Code: 023560

Case Type: C.I.

Processing Method: Fresh, qual

Number of Samples Received and Sample Type: 60 / sample case type?

Additional Comments:

PTD Sample Tracking Form

Date Samples Cooked and Initials: 20NOV18 VV

SOP#/Version Used: WD-020 Rev. 1. 11/01/15

Pepsin Date(s) and Initials: †Picked 26NOV18 VV

SOP#/Version Used: WD-021, WD-022 Rev. 1 11/01/15

Trypsin Date(s) and Initials: 05DEC18 W

SOP#/Version Used: WD-023 (Rev. 1 11/01/15)

Sucrose Date(s) and Initials: Not Req. @ time

SOP#/Version Used: WD-024 (Rev. 1 11/01/15)

Date Samples Read and Initials: 05DEC18 W

SOP#/Version Used: WD-005 (Rev. 1 11/01/15)

Date Volumes Read and Initials: N/A (Not Res.)

SOP#/Version Used: N/A

Date Results Emailed and Initials: N/A (OR count)

SOP#/Version Used: N/A

Training/Authorization Records: None - VV grandfathered into PTD.

Proficiency Tested: Yes (No)

If Yes, Date:

Number of Samples Prepared: 60

Date closed + Init: 05DEC18 W

Reagent Lot Numbers Recorded: Not Required at the time.

Media Logs Reviewed: Did not start media log until 2019.

Equipment Temperature and Calibration Logs Reviewed: WD 11/20 - 12/05: 9 days Recorded. WD-004 (TRYP fridge) high: 6.7 low: 5.4, WD-005 (Read fridge) high 5.5, low 4.7, WD-006 (FREEZER TRYP) high 18.2, low 22.6, WD-013 (PICK) high 3.5, low 3.2, WD-017 (PEPSIN) high: 5, low 3. WD-017 had some issues in the months leading up to Nov going as low as 0 at times. The temp was raised - no temp range on sheet @ time.

Additional Comments:

Spore Count Analysis Form

Not used on this case - tracking form*

Date Case Closed and Initials:

Reporting SOP#/Version Used:

Training/Authorization Record:

Does Report Accurately Reflect the Following Information:

* has been updated to include a #1-12 sample tracking table.

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Pepsin Trypsin Digest Samples

Date: 04/30/20

Auditor: VV

☐ # of Samples Received ☐ Test Performed ☐ Results for Each Sample

☐ Name of Report ☐ Pagination/End ☐ Dates Tested

☐ Were Results Accurately Transferred to Case Record

Additional Comments: SOPs were recently updated & all forms have been updated as of OCT 2019.

Cleaning Records: 2018 WD Nov 2nd all but trash, 9th all, 16th all minus sweep, 23rd all, 30th all but sweep.

No scale calibration OR autoclave logs used @ this time.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

Further Audits Required to Complete CAP and Schedule/Deadlines:

This form needs updated.
Review 01AUG20:
Update Cleaning Records to make bleach bath + waterbath dates easier to Record!

Reviewed by Laboratory Director (date and signature):

Reviewed by QA Coordinator (date and signature):

Filed (date and initial):

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Fish Health Inspection Certificate

Date: 05/12/2020

Auditor: VICTORIA VINCENT

Completion of Fish Health Inspection Certificate

SOP#(s)/Version Used: AD-005, 004 (Filing Reports and Fish Health Insp. Certs)

Training/Authorization Record for Sample Collection: Rev. 1. 28JAN2016 None used @ the time.

Additional Comments: Admin SOPs are being updated soon, and admin training forms must be generated.

FISH HEALTH INSPECTION CERTIFICATE

CPW Case Number: 18-224 USFWS Case Number: N/A

Name of Fish Source and Address/Location: Roaring Judy SFH, ISO 2 water code: D2356

Name of Owner or Manager: Seth Firestone 970-641-0190 seth.firestone@state.co.us

Inspection Dates and Classification: 11/19/18 (SPF), 02/06/18 (SPF)

No 2019 Report generated.

Collector: Evan Jones

Date collected: 11/19/2018 Date received: 11/19/2018

Is Sampling Location: ☒ Public ☒ ~~Culture Facility~~ Hatchery ☐ Other :

☐ Private ☐ Free-ranging Type of Water Supply: Well

Fish Pathologist Signature and Date: John Brennan 02JAN19

Certifying Official Signature and Date: Vicki Milano 12/21/18

Additional Comments: Delay in generation/signing of cert most likely due to holiday.

FISH EXAMINED

Lot No.: 1 Species: CUT Age (Mo.): 5 No. in Lot: 10,895

Obtained as Eggs (E) or Fish (F) from: RJ218HCC071 RJ2

Lot No.: Species: Age (Mo.): No. in Lot:

Obtained as Eggs (E) or Fish (F) from:

Lot No.: Species: Age (Mo.): No. in Lot:

Obtained as Eggs (E) or Fish (F) from:

Continue on last page as needed for all lots listed — not req.

Pathogens Inspected for and Results:

Lot No.: 1 Columns with Sample Numbers and Results: BF: 60/+, BR: 60/−, BK: 60/−, WD: 60/−, IPNV: 60/−, VHSV: 60/−, IHNV: 60/−, OMV: 60/−. Lab* all designated (A).

Lot No.: Columns with Sample Numbers and Results:

Aquatic Animal Health Laboratory
Colorado Parks and Wildlife

Vertical Audit of Diagnostic Accessions
Fish Health Inspection Certificate

Page: 2 of 2
Date: 05/12/2020
Auditor: VV

Lot No.: _____ Columns with Sample Numbers and Results: _____

Continue on last page as needed for all lots listed – not req.

Additional Comments: Remarks: None.

No VV We do not list (E) or (F) on certificates. Discuss change or removal of this phrase from certificates.

Corrective Action Plan #s (CAP #s) Initiated As a Result of This Audit:

Further Audits Required to Complete CAP and Schedule/Deadlines:

Admin SOP update progress:

Admin training forms progress:

Reviewed by Laboratory Director (date and signature): _____

Reviewed by QA Coordinator (date and signature): _____

Filed (date and initial): _____

No corrective actions plans were generated, however, a follow-up audit is required to ensure adherence to certain recommendations:

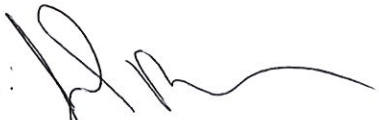
- Develop and implement an SOP change-tracking sign-off sheet that personnel sign.
    - VV- This will be quick, we can do as we edit each group of SOPs.
- Training documents for AI and AD SOPs must be generated (FD as well).
    - This will be fairly quick but we need to have ADMIN edited before we make it. AI we can generate as we edit the SOPs.
- VI-3.06 Needs to include steps on how to report results (how to properly fill out paperwork).
    - This will be addressed next time we edit this SOP.
- Need to decide if we want virology samples to have the same traceability as our PTD and BACT samples.
    - Decided as a group that, for now, what we do is fine.
- BACT-3.12 needs to be edited to include a step to note on the case paperwork when there's no growth on culture samples.
    - Will be completed the next time SOPs are edited.
- Batches of SKDM are not always tested as we don't always have live cultures of *R. salmoninarum.*
    - It's hard for me to keep alive, maybe I'm doing something wrong or maybe we just freeze a bunch back the next time we get growth.
- We need to include BACT media batch numbers on case paperwork, and add a section to media log to include preparation of DFAT conjugate information and a spot to record this on the paperwork.
    - Will do the next time we edit this SOP.
- As part of the training process in BACT, individuals are given a proficiency test. This procedure is not outlined, or recorded. This highlights a lack of standardized training protocols.
    - Training SOPs will take some time, I think we could have a rough draft in place by the end of August.
- Update cleaning records for PTD to make recording bleach bath and water bath dates easier to record/read.
    - Will do the next time we are finalizing PTD SOP edits.
- Certificate states: "Obtained as Eggs (E) or Fish (F) from:" but we don't list E or F for hatcheries. Change this phrase?

- From what I understand we're going to look at what other states/agencies are putting on their reports, and then go from there.

- Create/update a master list of qualified internal controls.
  - This can be an assigned task, due by the end of August. John has a list of frozen isolates/samples/controls that we could use to get started. From there it's just a matter of creating a file we can access with the following information:
    - Case #, Sample #, Location, (other information as applicable to control, as with the aforementioned information we can reference collection reports and other documents for further information as needed).
    - The pathogen/assay this control is/is meant to be used for.
    - Methods of presumptive and confirmatory testing, and the laboratory that performed each test.
    - Location of stored control.

Review of progress will be done August 2020.

Lab Director:  Date: 5/18/2020

QIA Coordinator:  Date: 18 MAY 20

(+co-coordinator)  Date: 18MAY20

As a result of the 2020 Internal Audit, Corrective Action 20.02 was generated, and the following changes were made:

- Develop and implement an SOP change-tracking sign-off sheet that personnel sign.
    - Done for each set of SOPs – 25SEP20.
- Training documents for AI and AD SOPs must be generated (FD as well).
    - Completed 25SEP20.
- VI-3.06 Needs to include steps on how to report results (how to properly fill out paperwork).
    - Completed September 2020.
- Need to decide if we want virology samples to have the same traceability as our PTD and BACT samples.
    - Decided as a group that, for now, what we do is fine.
- BACT-3.12 needs to be edited to include a step to note on the case paperwork when there's no growth on culture samples.
    - Will be completed the next time SOPs are edited.
- Batches of SKDM are not always tested as we don't always have live cultures of *R. salmoninarum.*
    - We make SKDM infrequently enough that taking from frozen stock is acceptable. When we reach fewer than 6 vials of frozen bacteria, the stock must be regenerated via culture.
- We need to include BACT media batch numbers on case paperwork, and add a section to media log to include preparation of DFAT conjugate information and a spot to record this on the paperwork.
    - Will do the next time we edit this SOP (2021).
- As part of the training process in BACT, individuals are given a proficiency test. This procedure is not outlined, or recorded. This highlights a lack of standardized training protocols.
    - Training SOPs and Proficiency Test SOPs done – September 2020.
- Update cleaning records for PTD to make recording bleach bath and water bath dates easier to record/read.
    - Completed September 2020.
- Certificate states: "Obtained as Eggs (E) or Fish (F) from:" but we don't list E or F for hatcheries. Change this phrase?

- What we do now is acceptable per group discussion.

- Create/update a master list of qualified internal controls.
  - Completed August 2020.

CAP #:	20.02	Date and Time of Nonconformity:	JUN 29.2020	Location:	Training documents

Define the Nonconformity Leading to this CAP:

	Nonconforming Work		Departure from Policy/Procedure
X	Audit Deficiencies		Proficiency Test Failure
	Complaint(s)		Equipment Failure
	Other:		

Event Description and Time Line of Events

Training documents not fully filled out. Trainer name not listed on every page, some empty signature lines.

Individual Responsible for this CAP

Victoria Vincent

Action Taken to Correct Immediate Problem (Description, Name, Date)

Victoria Vincent - meet w/ all staff & notify of nonconformity. Request if using training documents they are fully completed & full.
23JUN20

Identify Root Cause of Nonconformance (Use Fishbone Diagram)

Root cause = no training SOPs. Need to develop.

List of Possible Corrective Actions - Select the Plan that Eliminates or Greatly Reduces Recurrence of Nonconformity

1.) Develop training SOPs

2.) Modify training documents to be more user friendly & less tedious.

Implementation of Corrective Action: Project Plan, Budget (if applicable), Assigned Responsibilities, Deadlines

Training SOPs developed by OCT20.
↳ Implemented 25SEP20.

Training documents will be modified as the next round of SOP edits take place - 2022.

Detailed List of Actions Taken

SOPs AD 10.01 - SOP Training and AD-10.02 - SOP Editing were written and implemented.

Type of Review Required to Monitor Effectiveness

X	Ongoing Quality Control	Date of Review:	12OCT20
	Proficiency Testing	X	CAP Successful
	Internal Audit		CAP Unsuccessful
	Management Report		

Required Action Upon Review:

Continue to monitor use of training SOPs & documents. Ensure training docs are edited next SOPs are edited.

Investigated by: Victoria Vincent Date: 19OCT20

Approved by: _____ Date: _____