Appendix B – Additional Information Regarding Biosafety Level 2 (BSL-2)

Biosafety requirements are designed to protect humans, animals, and the environment. Diagnostic laboratories receive clinical specimens where the infectious nature of the specimen is unknown. AFS-FHS Tier 2 Laboratories must establish standard procedure to ensure the safety of their employees and prevent the accidental release of infectious material. Universally accepted guidelines for placing aquatic pathogens that only infect aquatic species or for pathogens that overlap and cause disease in animals or humans do not exist. However, CDC-NIH biosafety guidelines for infectious agents have been adapted for working with animal pathogens by different groups. For the purpose of the AFS-FHS laboratory Quality Control Recognition process, the following definitions will be used.

- Biosafety: prevention of exposure to hazardous disease agents or biological products that are capable of producing illness in human beings. These preventions can be adapted to protect specific groups of living organisms (i.e. fish, crustaceans, mollusks, corals, plants).
- Biosecurity: controlling the spread of disease agents or hazardous biological products to susceptible hosts.
- Biocontainment: preventing the release of a disease agent or hazardous biological product (key word is release).
- Biosafety Levels: are the combination of practices, safety equipment, and physical barriers.

Aquatic diagnostic laboratories work primarily with moderate-risk agents associated with aquatic animal disease of various severities. Specimens may also harbor unknown pathogens of moderate-risk that are associated with human disease.

- Moderate Risk: pathogens that regularly affect animals in a particular location, season, or in a particular species and where control programs that could limit spread are possible.

All AFS-FHS Tier 2 Laboratories must provide evidence that their established procedures meet or exceed biosafety level 2 recommendations according to the minimum guidelines/checklist provided at the end of this document. Additional resources and references for biosafety level 2 verification can be found at the following locations:

- USDA/APHIS - Veterinary Medical Officer (VMO) for your area
  - https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/contact-us
- https://www.cdc.gov/labs/BMBL.html
- Palic, D., et al. (2015). Biocontainment Practices for Coral Disease Research. Diseases of Coral. C. M. Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter and S. B. Galloway, Wiley-Blackwell: 442-488.

● Rusk, J. S. (2000) Biosafety Classification of Livestock and Poultry Pathogens, In C. Brown and C. Bolin, (Eds.). Emerging Disease of Animals, (pp. 13-22). Washington, DC: ASM Press.

Biosafety Level 2 Checklist				
Section	**Standard Microbiological Practices**	**Yes**	**No**	**Comments**
A1	Policies are in place that control access to the laboratory and these policies are enforced.			
A2	Hand washing is required, after handling potentially hazardous materials, after removing gloves, and before leaving the laboratory			
A3	Eating, drinking, smoking, inserting or removing contact lenses, applying cosmetics, and storing food or beverages for human consumption is not permitted in the laboratory area.			
A4	Mouth pipetting is prohibited; the mouth should not be used as an extension of hand for holding pen or other items.			
A5	Policies for safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed, implemented, and enforced.			
A6	Procedures are in place to minimize the generation of splashes and aerosols			
A7	Work surfaces are decontaminated after completion of work and after any spill or splash of potentially infectious material with an appropriate disinfectant.			
A8	Policies are in place and enforced that specify proper decontamination steps that must be completed prior to disposal of any cultures, stocks, or other potentially infectious material.			

A9	Signage: The universal biohazard symbol is posted at all entrances to the laboratory where infectious agents are handled. Posted information must include the laboratory's biosafety level, the names of responsible personnel, telephone number, and required enter and exit procedures. Agent information should be posted per the laboratory's policy.			
A10	A pest management program is in place and enforced.			
A11	All laboratory personnel receive appropriate training regarding their duties that includes instruction on necessary precautions to prevent exposure when working with potentially or hazardous material. Annual refresher training is required or additional training provided when policies change.			
A12	Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.			
	Special Practices			
B1	All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.			
B2	Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for			

	agents handled or potentially present in the laboratory.			
B3	Each institution should consider the need for collection and storage of serum samples from at-risk personnel.			
B4	A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.			
B5	The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.			
B6	Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.			
B7	Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.			
B7a	Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.			
B7b	Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.			
B8	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.			
B9	Animal and plants not associated with the work being performed must not be permitted in the laboratory.			
B10	All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a biosafety cabinet (BSC) or other physical containment devices.			
	Safety Equipment (Primary Barriers and Personal Protective Equipment)			
C1	Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:			
C1a	Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.			
C1b	High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.			
C2	Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library, and administrative			

	offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution.			
C3	Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.			
C4	Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:			
C4a	Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.			
C4b	Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.			
C4c	Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.			
C5	Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.			

	Laboratory Facilities (Secondary Barriers)			
D1	Laboratory doors should be self-closing and have locks in accordance with the institutional policies.			
D2	Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.			
D3	The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.			
D4	Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.			
D4a	Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.			
D4b	Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.			
D5	Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.			
D6	BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.			

D7	Vacuum lines should be protected with liquid disinfectant traps.			
D8	An eyewash station must be readily available.			
D9	There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.			
D10	HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.			
D11	A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).			