Model Quality Assurance/Quality Control Program For Fish Health Laboratories

American Fisheries Society Fish Health Section

I. **Introduction**

The purpose of the QA/QC Program is to ensure the quality, reproducibility and accuracy of the information and results generated by a fish health laboratory (FHL) for fish health inspection and diagnostic assays. Diagnostic protocols provided in updated versions of *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens* (AFS Fish Health Section Blue Book) provide excellent standards by which the diagnostic laboratory can abide when conducting laboratory assays. Following a carefully written protocol, however, does not in itself assure that the consequence is a quality product. In order for a laboratory to assure quality services it must first establish a system of quality control by which every aspect of laboratory management, structure and upkeep are implemented. The purpose of this Model Quality Assurance/Quality Control Program for Fish Health Laboratories is to provide a structure by which laboratories providing fish disease diagnostic services can develop individual, lab-specific programs. The document is geared for adaptation at small laboratories as well as large establishments, and will serve to address many of the critical elements provided in the ISO/IEC (International Organization for Standardization/International Electrochemical Commission) International Standards 17025, as well as those standards published by OIE in (Office International de Epizooties). The present document has been adapted from the QA/QC Program established by the U.S. Fish and Wildlife Service within the *Handbook of Aquatic Animal Health Procedures and Protocols* (2004).

II. **Policy and Implementation**

A. Management Responsibilities - The FHL shall have a Director whose tasks include the following:

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

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

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

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

2. Depending on the management authority overseeing the funding, policy and direction of the FHL, the FHL Director shall be accountable for QA/QC concerns of the FHL to the management authority involved.

B. Personnel/ Personnel Responsibilities

1. Each FHL maintains an adequate number of employees for all functions. All employees possess the necessary education, training, technical knowledge, skills and experience for the tests conducted. In addition, all employees are free from pressure or inducements which might adversely influence their judgment or the results of their work.
2. Each employee shall be responsible for monitoring each test he/she is conducting to ensure that facilities, equipment, practices, and record keeping conform to this QA/QC Program.
3. Each employee shall take the necessary precautions to avoid contamination of the test, control, and reference substances.
4. Each employee shall follow established national safety and health regulations in the operation of each laboratory unit, handling of hazardous materials, and procedures for storage and disposal of hazardous wastes.

C. Quality Assurance Coordinator

1. The FHL shall have a Quality Assurance Coordinator whose responsibilities include the following:
   a. Implementing and monitoring of the QA/QC Program
   b. Implementing all necessary quality controls to ensure the accuracy and precision of reported data.
   c. Monitoring laboratory practices to verify continuing compliance with policies and procedures.
   d. Evaluating instrument calibration and maintenance records.
   e. Ensuring the validation of new technical procedures.
   f. With the Director, investigating technical problems, proposing remedial actions, and verifying their implementation.
   g. Providing recommendations for training to improve the quality of laboratory staff.
   h. Proposing corrections and improvements in the quality system.
   i. With the Director, cooperate with and accompany teams conducting external and/or internal QA/QC audits of the facility (See Appendix A).
2. Technical Qualifications Files: A technical qualifications file is maintained for each laboratory staff member. Technical qualifications files include the following items:
   a. A resume of qualifications, skills, experience, and certifications.
   b. References to all training classes, seminars, short courses, and conferences attended.

D. Laboratory Facilities

1. Sample Collection/Necropsy: All samples will be collected by qualified staff members of the fish health laboratory, under supervision of an AFS – Fish Health Section certified Fish Health Inspector or other qualified official. Necropsy and sample collection is performed in accordance with accepted protocols (Appendix D).

2. Sample Processing: Virological, parasitological, bacteriological, histopathological, immunological (ELISA) and molecular biological (PCR) assays shall be conducted according to accepted protocols (Appendix D). Labs which do not have particular capabilities ship necessary samples for analysis to a private, academic or government laboratory with appropriate capabilities. Condition and integrity of sample submissions upon arrival should be noted (Appendix C).

3. Laboratory Space and Design

   a. Fish Heath Labs should comply with national and local standards of health and safety at or equivalent to Level II Containment (for biocontainment standards see http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

   b. Labs should provide separated areas for administrative activities, fish handling, and laboratory testing.

   c. Each laboratory room is equipped with adequate space and environmental conditions including cleanliness to perform assigned tasks.

   d. Storage spaces are adequate to maintain equipment, supplies, samples, and chemicals without danger of cross contamination.

E. Laboratory Equipment and Supplies

1. Equipment – The assigned Quality Assurance Coordinator (QAC) is responsible for maintenance of all laboratory equipment, and recording all maintenance records in a common log:

   a. The QAC ensures that all microscopes, balances, pipettes, thermometers, meters, incubators, refrigerators, freezers, hoods, spectrophotometers, and other instruments in use are calibrated and maintained on a routine basis by laboratory staff using the equipment (Appendix E).

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

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

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

   e. Equipment used for generating measurements is calibrated and/or standardized according to recommendations provided in Appendix E.

2. Reference stocks and reagents - The QAC is responsible for assuring that all reagents and reference stocks are maintained under proper storage conditions, labeled and handled appropriately by all laboratory staff:

   a. All reference stocks shall be retained with original labels from the supplier or are labeled by name, date of receipt, chemical abstracts number (CAS) or code number,

batch number, expiration date if perishable, and include National Fire Protection Association (NFPA) labels indicating safe use and storage requirements.

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

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

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

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

f. Current Material Safety Data Sheets (MSDS) are available to employees for all chemicals used, as required by the "Right to Know Law".

g. Necessary personal protective equipment and training in equipment use is provided to all laboratory personnel.

h. Fish Health Labs should have a "Safety and Chemical Hygiene Plan". All laboratory personnel are to utilize equipment and reagents in compliance with this plan.

i. Mixture of substances - When test, control, or reference substances are mixed, the date of preparation, initials of the preparer, and the exact contents of the mixture shall be labeled on the bottle along with storage requirements and expiration date and proper NFPA labeling.

F. Chain of Custody/Case Tracking

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

a. The case history number uniquely identifies the test samples on receipt and tracks the case throughout the laboratory. Upon receipt the case number is assigned and labeled on all sample containers and racks following external decontamination.

b. The case history number, along with information pertaining to the specifics of the samples received, is recorded on either a Case History Record (CHR) cover sheet and/or in a Case Report book. The following information is to be included:

1) Case History Number

2) Date of Receipt

3) Date Sample Taken

4) Sample Site (including, where possible, GIS information)

5) Name of Sampler

6) Recorder Initials

7) Species and Age -class of fish

8) Condition of Samples at receipt

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

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

e. All tubes, bags, or other sample containers are labeled with chemical resistant laboratory markers with pertinent information to allow for accurate tracking of samples through each laboratory area.

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

2. Sample tracking in individual labs:

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

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

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

3. Record Retention

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

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

G. Standard Operating Protocols and Conduct of tests

1. Each fish health laboratory shall follow specific protocols depending upon the type of case being conducted (Appendix D).

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

b. Directors are responsible for approving other protocols prior to their use. Critical protocol deviations must be documented on the CHR with a description of the

procedures used, and/or citation from the literature.

2. Each employee conducts testing in strict accordance with established protocols as described in Section II.G.1 of these guidelines.

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

   b. Pertinent entries are dated and initialed by the employee performing the work.

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

H. Routine Quality Control Procedures

1. Reagent Quality Control

   a. Controls: Known controls and standards are tested and compared along with the samples in question with every test conducted. Standards and controls are kept separate from samples when stored. Detailed information on controls, reagents and media used for each test is described in established protocols (Appendix D).

   b. Contamination Checks: On a routine basis and/or when a problem is suspected, materials and supplies used in sample testing are tested for contamination, using known controls.

2. Routine Quality Control in the Sample Processing/Necropsy Laboratory

   a. Sample Tracking

      1) All samples received in the sample Processing/Necropsy Lab are immediately assigned a CHR number as described in II.F.

      2) The container holding the samples is externally decontaminated (Appendix F) prior to opening. Upon opening of container, the inside of the container is inspected for sample spillage which would need further disinfection.

      3) Tubes, bags or other items containing tissue samples are removed, externally disinfected (Appendix F), and placed in clean laboratory racks, bags or containers bearing the appropriate CHR number.

      4) Data on each tissue sample is recorded and entered onto the CHR data sheet as described in section II.F. Any written material and sample descriptions received with the samples are attached to the CHR.

      5) Samples are distributed to respective laboratories for incubation, processing and/or archiving.

   b. Contaminant Control - The following are basic measures to be taken to insure contaminant control in any laboratory.

      1) Wear clean lab coats within laboratory.

2) Wash hands with antibacterial soap before and after lab work.

3) Clean countertops with an appropriate disinfectant (Appendix F) preceding and following any lab work.

4) Decontaminate container surfaces with 70% alcohol spray (Appendix F) before and after use.

5) Clean countertops, floors and waste cans routinely or when needed with an appropriate solution of disinfectant.

6) Clean inside surfaces of refrigerators with an appropriate disinfectant when spills occur.

7) Soak waste materials from sample processing/ necropsies in an appropriate concentration of sodium hypochlorite solution (Appendix F) prior to disposal, or autoclave prior to proper disposal.

8) Thoroughly decontaminate tube racks, slide boxes and other items with possible contamination prior to reuse.

9) Do not eat, drink or smoke in the lab.

10) Immediately clean and disinfect any biological agent or media spills.

11) In general, avoid clutter and maintain lab equipment in an orderly and functional manner.

c. Necropsy - Live or freshly dead animals are often received for necropsy at fish health laboratories. The following are general considerations for maintaining QA/QC of samples obtained during a lab necropsy:

1) Keep all specimens cold throughout necropsy procedure.

2) Disinfect working surface prior to necropsy.

3) Set up all equipment necessary prior to commencing dissection, including separate containers for decontaminating discard items and utensils.

4) Proceed with necropsy according to established protocols.

5) Following necropsy, disinfect all work areas, equipment, microscopes, etc. involved in examination of tissues.

3. Routine Quality Control in the Parasitology Laboratory

a. Sample Tracking

1) All samples received in the Parasitology Lab are to be labeled with the proper CHR number attached to the sample container (i.e. bag of heads, whirl paks containing tissue samples, etc.)

2) Laboratory Data Log – All incoming parasitology samples must be logged into a Parasitology Lab data logging system designed to record the number and type of samples, and field sampling date. The log must contain designated spaces for

logging each case out as it is completed, to include the date and initials of the employee completing the lab work.

3) Data Entry Forms – a form must be completed for all parasite assays and must include the CHR number, type of procedure used for analysis, and results.

4) All testing records must be initialed and dated by the employee performing the lab work.

b. Contaminant Control – In addition to those listed in Section II.H.2.b., the following are measures to be taken to insure contaminant control in the parasitology lab:

1) Gloves and lab coats must be worn when handling and processing any parasitology samples.

2) Hands must be washed after removing gloves.

3) All parasitology lab waste must be autoclaved for a minimum of 20 minutes at 121^{o}C when disposing of waste materials exposed to all parasites. Heat disinfection is also effective for controlling myxosporean parasites as spore viability can be reduced to 0% with temperatures of 95^{o}C for 15 minutes (Turner, et al 1999). (When autoclaving large volumes of lab waste, time may need to be increased to insure that the core temperature of waste reaches 121^{o}C and is maintained for a minimum of 15 minutes).

4) Whenever possible, use disposable laboratory materials for assays to prevent contamination of lab ware (such as disposable paint filters for filtering pepsin-trypsin digest preparations).

5) Glassware, processing lab ware and other non-disposable or reusable lab materials must be soaked in a minimum concentration of 5000 ppm sodium hypochlorite solution for at least 10 minutes (Appendix F)

6) Store all clean glassware, equipment and reagents in designated areas free from exposure to parasite samples

c. Cross Contamination Control

1) Assays must be performed at designated parasitology work stations.

2) Work stations must be decontaminated prior to and after each lot of each case history with 5000 ppm chlorine solution (Appendix F).

3) Cover work areas with bench paper and change with each lot of each case history.

4) Work on one case history at a time, separating individual lots to prevent cross contamination between lots within a case history. Work areas, glassware and equipment used for assays must be disinfected between each lot of each case history.

d. Reagents

1) A chemical inventory must be maintained of all reagents used in the Parasitology

Lab to meet the standards outlined in section II.E.2. (Reference stocks and reagents).

4. Routine Quality Control in the Bacteriology Laboratory

a. Sample Tracking

1) All samples received in the Bacteriology Lab are to be labeled with proper CHR number attached to all racks containing culture tubes, and written on all Petri plates containing media.

2) Laboratory Data Log - All samples must be logged into the Bacteriology Lab data logging system designed to record the number and type(s) of samples, and the date each sample was taken in the field. The log must contain designated spaces for logging each case out as it is completed, to include the date and initials of the employee completing the lab work.

3) Data entry forms - for all assays conducted on each case, a form must be completed which contains at a minimum the CHR number, each sample identification number as well as description and results of all biochemical, morphological, and serological tests conducted and test results.

4) Daily testing records for each case must be initialed and dated by the employee performing the lab work.

b. Contaminant Control - In addition to those listed in Section II.H.2.b., the following are measures to be taken to insure contaminant control in the bacteriology laboratory:

1) Wash hands with antibacterial soap before and after lab work or spray with alcohol solution (Appendix F).

2) Dispose of used media (Petri plates and tubes) by autoclaving at 121^{o}C for 20 minutes. Media can then be safely discarded with the trash.

3) Glassware tops should be covered with foil prior to proper sterilization. Foil sheets to be used should be checked for pin-holes and tears, and proper fit must be ensured.

4) Soak depression-glass slides used for hanging drop motility assays in chlorine solution (Appendix F) prior to cleaning with laboratory grade detergent.

5) Soak used disposable loops, pipettes and other expendable items which become contaminated in chlorine solution before discarding in the trash.

c. General Aseptic Technique - Aseptic technique is necessary to avoid both infection and cross-contamination of microorganisms between the body and cultures of bacteria which are handled daily in the Bacteriology Lab. The primary goal of aseptic technique is to prevent the introduction of undesired microorganisms into the media and samples to be tested. The following includes materials and guidelines to ensure this goal is met:

1) Loops: Use sterile disposable loops whenever possible. Remove loops from

packaging just prior to use and place in chlorine solution or hazard bag to be autoclaved immediately following use. Transfer of bacteria with loops or sterile swabs must be completed rapidly. At no time should the loop come in contact with any object other than the bacteria and the medium it is being transferred onto or into. If this should happen, dispose of the loop and get a new one to complete the transfer. Metal loops should be properly sterilized by heating to red heat between transfers. For sterilization of a used metal loop, it should be lowered carefully into the flame to prevent splattering live organisms in the lab.

2) Avoid talking, coughing and sneezing when bacterial cultures and media are exposed to the air.

3) Petri Plates: Prior to use, the medium in each plate should be examined for possible airborne contamination which may have occurred during media preparation and/or storage. When working with plates, minimize the amount of time the lid is opened and the degree the lid is lifted.

4) Test Tubes: When working with tubes, open only one tube at a time, using the little finger of the loop hand to unthread and hold the cap (threads facing downward). Keep the time the cap is removed from the tube to a minimum.

5) Pipettes: Utilize sterile pipettes and pipette aids when transferring media, media components, serological supplies or bacterial cultures. Never pipette by mouth.

d. Cross-Contamination Control - In addition to the measures listed above, the following guidelines are followed to avoid cross contamination of microorganisms between case histories:

1) Each work station should contain only one case history at a time.

2) Work stations should be decontaminated thoroughly between cases (Appendix F).

3) Stocks of sterile media and sterile supplies are kept separately from areas of the laboratory where live organisms exist (incubators and workstations).

4) All tubes and assay containers from one case are to be racked separately from other cases, and labeled clearly with the CHR number.

e. Media and Reagent Preparation:

1) When working in a laminar flow hood allow the blower fan to operate at least 10 minutes prior to working under the hood. Spray the entire work surface and any materials to be used with 70% isopropyl alcohol or ethanol (Appendix F). Exposure of surfaces to UV light for 10 minutes before and after use is recommended for additional protection.

2) Media which require exposure while drying, such as agar plates, should be allowed to cool and harden within the aseptic environment of the laminar flow hood. Media should not be exposed to excessive amounts of ultra-violet light, as this can degrade some of the ingredients. **Warning:** Exposure to the UV light can cause skin and eye damage! Turn the UV light off whenever placing objects or

working in the hood!

3) All reagents are transferred and prepared using aseptic technique.

4) All filter decontamination and dispensing of media and reagents is performed under a decontaminated laminar flow hood. A medium which is distributed to containers prior to autoclaving does not need to be dispensed under aseptic conditions.

5) All new lots of media and batches of reagents for biochemical testing of live bacterial cultures are tested using live bacterial control cultures maintained within the laboratory. Control cultures are obtained from American Type Culture Collection (ATCC) or from one of the reference labs listed in Appendix C.

6) All new batches of serological reagents are tested using both positive and negative controls.

7) All serological testing on sample material is performed side by side with positive and negative control material (cultures or tissues).

8) Identification and Confirmation of bacterial pathogens - it is important that identification of a bacterial agent be confirmed using specific techniques recommended in the appropriate Blue Book chapter, or by accepted protocols listed in Appendix D.

9) Note: laboratories utilizing live control organisms must follow appropriate containment levels according to the status of any agents considered to be exotic to the region the laboratory is located (for biocontainment standards see http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm)..

5. Routine Quality Control in the Virology Laboratory

a. Sample Tracking

1) All samples received in the Virology Laboratory are labeled with the CHR number attached to all relevant equipment (e.g. racks) containing sample material. CHR number is transcribed to all additional racks and micro titer plates used for processing that case through the lab.

2) Laboratory Data Log - All samples are logged into the Virology Lab log upon receipt in virology lab. The log is designed to record the number and type of samples, and the date each sample was taken in the field. The log must contain designated spaces for logging each case out as it is completed, to include the date and initials of the employee completing the lab work.

3) Data entry forms - for all assays conducted on each case, a form must be completed which contains at a minimum the CHR number, each sample identification number as well as description and results of all assays conducted.

4) Read micro titer plates every at least three alternating days per week, and on days

when case histories are to close. Record and initial observations in the virology log and on the CHR. Indicate cell condition, presence or absence of contamination, toxicity or any apparent viral cytopathic effects (CPE), and condition of controls, including progression of CPE in virus positive controls.

5) Confirmation and identification of viral CPE – it is important that identification of a viral agent be confirmed using techniques recommended in the appropriate Blue Book chapter, or by accepted protocols listed in Appendix D.

6) Upon completion of the viral assay for a particular case history, results and dates are recorded on the data entry form and the CHR form. Also, append the data entry form to the CHR form.

b. Aseptic Technique - The goal of aseptic technique is to prevent the introduction of undesired microorganisms into the viral assay system. The goal of contaminant control is to prevent cross contamination of samples and to destroy any microorganisms present in samples, containers, or supplies prior to their disposal. The following steps will be taken to insure both aseptic technique and contaminant control:

1) When working in a laminar flow hood allow the blower fan to operate at least 10 minutes prior to working under the hood. Spray the entire work surface and any materials to be used with 70% isopropyl alcohol or ethanol (Appendix F). Exposure of surfaces to UV light for 10 minutes before and after use is recommended for additional protection.

2) Glassware stored in the virology lab first must have been rinsed thoroughly with tap water, rinsed three times with tissue culture grade water, covered and autoclaved (minute amounts of residual detergents and reagents can adversely effect tissue culture). Glassware used for harsh chemicals (chlorine, acetone, HCl, etc.) should be labeled as such and kept separate from glassware used for cell culture components. Use laboratory glassware detergent when necessary and rinse thoroughly.

3) Sterility checks must be done on media prior to addition of antibiotics. When contamination is detected, all suspect media should be discarded and each media component should be checked separately to determine the source of contamination.

4) Sterility checks involve inoculation of tryptic soy or brain heart infusion broth under the sterile hood and checking for bacterial growth for 48 hours. Sterility checks should be completed before use of media components and before adding antibiotics to complete media. Documentation of sterility checks for each batch of medium prepared should be kept on file.

5) Anything that comes in contact with samples or known virus must be disinfected or autoclaved before reuse or disposal.

6) Chlorine solution used for disinfection should be changed weekly (see Appendix F for procedures and alternative disinfection).

c. Media and Reagents

1) All chemicals, reagents and media components must be dated upon receipt in the virology lab. Whenever media, reagents or antisera are prepared, label with the date of preparation, content, and other information as described in II.E.2 (Reference stocks and reagents).

2) Each bottle, tube, flask or plate in a virology refrigerator or incubator must be properly labeled or will be discarded.

3) An inventory log will be maintained and updated on a bimonthly basis. The inventory log is primarily for much-used disposable supplies, but other supplies such as media components, balanced salt solutions, and versene salts must also be monitored regularly.

d. Cell Culture Stocks - Maintenance of healthy tissue cultures is imperative for all virology assays. The following are procedures routinely employed to ensure that healthy, sensitive cultures are used for each assay:

1) Prior to use, check each culture for possible contamination, the appearance of the cell sheet, and the pH of the medium. If the medium is turbid, bacteria or yeast are probably present. Cotton-like tufts indicate fungal contamination. Do not use contaminated cultures. Discard all suspect cultures and check into the source of contamination.

2) Examine cells microscopically to make certain that cells are healthy and the sheet is confluent.

3) Allow recently thawed versene/trypsin solution (V/T) to warm to room temperature (15-20^{o}C). If too warm, trypsin, as an enzyme, may work too quickly and damage cells or cause clumping.

4) Label new flasks with cell line, new passage number (old number plus one), medium lot number, and date.

5) Cell lines for virology are sent to a reference laboratory for sensitivity testing and mycoplasma contamination checks according to Appendix B-4. Cell lines can be obtained from sources listed in Appendix C. Use of the most sensitive cell lines for the viruses to be detected is encouraged.

6. Routine Quality Control for the Immunoassay Laboratory (ELISA assay used to detect *Renibacterium salmoninarum* or other pathogens that may apply)– The ELISA technique discussed in the following section was developed and standardized by researchers at the USGS Western Fisheries Research Center in Seattle. Refer to Blue Book Bacterial Kidney Disease Methods Appendix 4 (1.14.A4) for controls and Appendix 5 (1.14.A5) for the detailed assay procedure. This ELISA uses reagents and antibodies presently produced by Kirkegaard and Perry Laboratories (http://www.kpl.com). It is a highly sensitive and complex protocol which requires careful preparation of assay reagents and strict adherence to a detailed protocol. Several steps are included in the overall methodology of ELISA to ensure quality control of reagents, consistent test results, and optimum performance of the

assay, and are included in appendices to the protocol. **There are other ELISA systems which are commercially available and approved by USDA for certain fish pathogens. These kits must be used according to specific manufacturer's guidelines.**

a. Tissue collection and processing – fish tissues are collected and processed with care to avoid contamination of kidney tissue with foreign material or gastrointestinal contents (fish feed) which could cause false-positive reactions in the ELISA. Sterile, individual instruments are used for each tissue collected to prevent cross-contamination between positive and negative kidney tissues.

b. Sample Tracking - it is important that all individual samples or pools of samples processed for ELISA be recorded and tracked.

   1) The CHR number, individual sample and pool numbers, controls and blanks are assigned to a particular well on the 96 well ELISA plates prior to setting up the assay. These are recorded on an ELISA template data sheet, and should be referenced during loading of samples and control materials onto each plate to ensure accuracy of results.

c. Checkerboard titration (optimization of antibody dilution)- the checkerboard titration assay is performed first to test antibody reagent quality and ensure optimum assay performance. Antibody testing, using a dilution matrix, allows the optimum antibody dilution to be determined for a specific lot of antibody reagents. This step ensures consistent ELISA results over time regardless of potential changes in antibodies supplied by the manufacturer. Follow the checkerboard titration assay as described in established protocols (Appendix D).

d. Standardization of antibody reagents: optimum dilutions are determined for the coating antibody (CAb) and horseradish peroxidase-labeled secondary antibody (HRP-Ab) by checkerboard titration assay. Once optimum working dilutions are established, antibody reagents are pooled and aliquoted into small working volumes. Aliquots are frozen for future use.

e. Performing the assay: the assay is performed following an established protocol (Appendix D). Special care is taken to ensure accurate dilution of antibody reagents, placement and isolation of control wells on each plate, and adherence to precise incubation periods. Dedicated equipment such as pipetters, reagent dispensing cassettes, and glassware are additional precautions that are taken to ensure accurate and consistent test results. Controls: established guidelines for the control plate, control wells on each subsequent plate, and the application of the standardized Negative Control (NC) are included in the protocol to ensure optimum assay performance and consistent data analysis. One full control plate is included for every five (5) plates in an ELISA run. The control Plate has at least two replicates of the following reagents:

   1) Positive Control – Positive antigen preparation.

   2) Negative Control - Negative Kidney tissue, tested by ELISA and PCR

   3) Blank - Wells receive PBS-T20 diluent only and serve as a Blank negative control.

4) Conjugate Control - Wells receive Coating Solution without Antibody and serve as control for non-specific binding of the HRP-conjugated Ab to the well surfaces or the Coating Antibody.

5) Substrate Control - Wells receive Milk Diluent without HRP-Ab and serve as the control for non-enzymatic production of the ABTS color reaction.

6) Each subsequent plate also contains one column of control wells: 4 wells of Negative Control tissue and 4 Blank wells. The first Control Plate and control wells on subsequent plates allow close monitoring of assay performance between plates during a single assay, and between assays performed on various dates.

f. Positive threshold: for data interpretation, an appropriate threshold is determined according to the desired sensitivity and particular group of samples being tested. For example, two standard deviations above the mean OD value of the Negative Control are used to establish the positive threshold used for samples collected by fish health labs of the U.S. Fish and Wildlife Service. The Negative Control tissue consists of salmonid kidney tissue tested by ELISA and PCR and found to be negative for *Renibacterium salmoninarum* antigen or DNA. The mean OD value of replicate sample wells is compared to the threshold value to determine the positive or negative status of a test tissue..

g. Annual testing program: a Quality Control program for ELISA among fish health laboratories should include an annual testing program. Sample sets with known OD values are received from an appropriate reference laboratory and tested. Results are summarized and any variation from acceptable ranges will be investigated for the source of variation and corrective steps will be taken. An annual testing program will ensure consistent and comparable test results between various fish health laboratories in North America.

7. Routine Quality Control for the Molecular Biology (PCR) Laboratory - The Polymerase Chain Reaction[1] technique (PCR) is currently used in many FHLs to corroborate results of screening procedures. PCR employs oligonucleotide primers to amplify base pair segments of genes specific for the target pathogen. Reverse Transcriptase-PCR (RT-PCR) employs an initial reverse transcriptase step so that complimentary DNA can be amplified from viral RNA. DNA or RNA is extracted from various fish tissues and laboratory assay products, such as cell culture supernatant containing viral agents, and amplified using forward and reverse primer sets. In some instances, the amplified product is re-amplified using an additional "nested PCR" technique. The DNA products are then visualized by gel electrophoresis. Detailed PCR procedures for each target pathogen are followed as written in accepted protocols (Appendix D). PCR Quality assurance and control procedures outlined below are extremely important when performing all PCR assays:

a. General Considerations:

1) Work surfaces should be decontaminated by washing with 10% chlorine bleach to

[1] PCR is Patented by Hoffman-LaRoche, Inc.

hydrolyze possible DNA contaminants. Likewise, tube racks should be soaked in 10% chlorine for 30 minutes prior to use. Ultra violet decontamination is also recommended where possible.

2) Store RNA and DNA samples and templates separately from PCR reagents.

3) Employ aerosol resistant pipette tips and/or positive displacement pipetters during all extraction and amplification procedures. Separate pipetters should be dedicated for use with reagents only and amplified products only.

4) One aerosol drop of amplified DNA contains thousands of strands of DNA which can easily contaminate reagents. Therefore, three separate areas of lab space are optimal to reduce the risk of contamination:

   i. Master Mix (MM) area with UV hood - for mixing and aliquotting master mix reagents. Supply area with dedicated pipetters, ideally positive displacement Pipetter/tips. No samples or amplified DNA is to be handled in or near this area!

   ii. Sample Preparation Area - for loading of extracted (template) DNA from samples (also with a UV hood).

   iii. Amplified DNA Area - supplied with pipetter dedicated for **amplified PCR product ONLY.** Handle any amplified PCR products in this area only, and clean area and equipment thoroughly with "DNA-away" type solutions after working with amplified DNA Use four separate work areas for RNA and DNA extraction, reagent preparation, sample loading, and PCR amplification. Separate reagent and sample loading work surfaces must be equipped with UV decontamination.

5) Change gloves frequently when handling samples during all procedures and dispose of used gloves immediately.

6) Dispose of trash containing amplified DNA products frequently.

b. Sample Collection - When tissues are collected directly from fish for PCR assays, samples should be collected on a clean bench-top which has been disinfected using a 10% chlorine solution if possible.

1) Use sterile collection utensils between each lot of fish tissue collected. If data from individuals is of concern, use separate utensils for each individual. **Alcohol will not effectively decontaminate DNA from utensils.** If individual utensils are not available, flaming metal utensils between sampled will effectively remove contaminants from previous samples.

2) Keep samples cold and freeze as soon as possible at -70^{o}C until processing can be accomplished.

3) RNA is extremely sensitive to enzymes existent in most sample tissues. Samples collected for RT-PCR should be frozen immediately, and transported on dry ice. An RNA stabilizing reagent can also be used and does not require that samples be

frozen immediately if manufacturer's recommendations are followed.

c. Extraction of DNA or RNA from samples - All general considerations should be employed including the following:

1) Use micro centrifuge tubes with sealed or locking lids so that contaminates do not escape during heating cycles that might cause breaching of lit seal.

2) Use the accurate amount of tissue suggested by the extraction kit manufacturers. If this is exceeded, proper lysis of tissues will not be accomplished.

3) Controls: all extractions should employ a negative control (sterile RNase/DNase free water, or negative tissue), and a positive control (known positive tissue) to be run along-side of unknown samples. These controls will allow for detection of contamination as well as assure that the extraction was successful.

4) Quantification of extraction product - it is advisable that extracted products be measured using a spectrophotometer to ensure that enough DNA or RNA was successfully extracted. Refer to individual protocols for guidance on the amount of DNA or RNA recommended for each PCR reaction and dilute as needed.

d. Running the PCR - All general considerations should be employed including the following:

1) Decontaminate and UV reagent and sample loading workspace both before and after use for at least 15-30 minutes (be aware that over-use of UV lamps can break down plastics).

2) Master Mix (MM): dispense water first and TAQ polymerase last (the TAQ is the most unstable of the ingredients in the MM). Mix ingredients well before dispersing aliquots, as the TAQ is stored in a glycerol-based buffer which sinks to the bottom of the MM tube.

3) All reagent batches should be marked and recorded for each test run so they can be checked if problems occur with the assay.

4) Primers: newly received primer batches should first be tested on known positive/negative controls.

5) Controls: prepare enough MM for extraction positive/negative controls AND PCR positive/negative controls (4 extra samples). The **extraction controls** will ensure that the extraction was successful and contamination did not occur. The **PCR controls**, using sterile water (negative) and known positive DNA from previous extraction (positive) will ensure that the PCR process was successful and that contamination did not occur.

6) Before loading into thermal cycler, give tubes a "quick-spin" to ensure that all reagents and sample are drawn down from sides of tube.

7) It is recommended that all cycles begin with a 2 minute pre-dwell at 94^{o}C. This allows for all DNA to denature into single stranded form at the beginning of

cycling, and reduces primer dimers and mis-priming.

8) After cycling, tubes may have a ring of condensation near rim of cap. Before opening tubes, perform a "quick-spin" to draw this fluid down into the reaction area of the tube and reduce the possibility of aerosol contamination upon opening tubes.

9) Document gel lane assignments for each sample and controls, and allow for at least one lane for a DNA ladder reference.

10) Photodocument all PCR gels and keep with the cases run (or provide reference for finding the photodocumentation.

III. **Laboratory Safety**

Copies of MSDS's (Material Safety Data Sheets) for all chemicals and reagents in the laboratory are kept on file and within easy access and viewing for all personnel. In addition, each laboratory maintains a Safety Manual and Chemical Hygiene Plan the safety manual specific for that laboratory. All personnel are to follow safety precautions published within MSDS's for each reagent used at the laboratory.

Zoonotic agents may be in exudates, tissues, and environmental samples (soil and water). Direct contact of skin or mucous membranes with infectious materials, ingestion, and accidental inoculation are the primary laboratory hazards associated with sampled material and cultures. Aerosols created during the manipulation of tissue homogenates also pose a potential infectious hazard to laboratory personnel.

Biosafety level 2 practices are recommended when working with clinical materials (tissues and bodily fluids). Laboratory personnel need not confine established cultures with low aerosol potential to an approved safety cabinet. Laboratory biosafety level 2 criteria are outlined in *Biosafety in Microbiological and Biomedical Laboratories*, 1999 U.S. Department of Health and Human Services, Public Health Services, Center for Disease Control, and National Institutes of Health, 4th edition, U.S. Government Printing Office, Washington D.C. (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm)

IV. **Review of Guidelines**

These guidelines are to be reviewed and amended on a regular basis by the AFS/Fish Health Section QA/QC Committee. Comments are to be submitted to the Committee Chairperson. Changes, and justification for no change, will be compiled and changes will be implemented during regular Blue Book revisions.

APPENDIX A
INTERNAL AND EXTERNAL AUDIT PROGRAM

I. Introduction

The goal of a Quality Control program is to assure that all of the data generated by a scientific laboratory be scientifically valid, defensible and of known precision and accuracy. To assure that the obtained data meets these criteria, both internal and external audits should be periodically conducted under the direction of the laboratory's Quality Assurance Coordinator and Director. The information acquired from the audits will be used to estimate the quality of the analytical data, to identify deficiencies, and determine and implement corrective action.

II. Purpose

Internal and External auditing will provide an evaluation and establish the effectiveness of QA/QC programs. The audits will ensure that the requirements outlined in the QA/QC program are followed to provide quality of analytical data in terms of objectives for precision, bias, representation, comparability, and completeness.

A. The Internal Auditing Plan requires a periodic laboratory review by the Quality Assurance Coordinator (QAC) to include:

1. field and sampling activities review
2. laboratory activities review
3. laboratory performance review

Results of the internal audit will be reported to the FHL Director for assessment and correction of the observed deficiencies.

B. An External Auditing Plan should require a periodic laboratory review by a qualified team of individuals who are not employed by the FHL to be reviewed. The purpose of this assessment is to provide insight to QA/QC deficiencies not detected by the internal audit plan. The results of the external assessment should be submitted to the FHL Director for assessment and necessary corrective action.

C. Check lists are provided for guidance in performing these reviews

III. Objectives (Internal Audit Plan)

A. Field and Sampling Activities Review: the review of field and sampling activities shall be conducted by the QAC or by one or more persons knowledgeable in the activities being reviewed including:

1. Completeness of field sampling reports: this review determines whether all requirements for field sample collections have been fulfilled as outlined in accepted protocols (Appendix D). Completed records shall be maintained for each collection including sample receipt and laboratory tracking.

2. Identification of Sample Integrity: this review evaluates field sample collections to assure samples were collected in accordance with accepted protocols (Appendix D). This will include:

   a. reviewing whether samples were collected under the direction of a qualified individual.

   b. identifying whether lethal and non lethal sampling were obtaining using humane methods by appropriate protocols (i.e. use of MS222 for euthanasia).

   c. evaluating whether appropriate tissue was collected for each sample assay.

   d. identifying whether appropriate preservation was used for collected tissues for transport to laboratory centers for processing.

   e. reviewing proper and timely shipment or transport of samples to laboratory centers for processing.

3. Validation of Field Analysis: this review will evaluate whether field data obtained meets QA/QC criteria including reviews documenting instrument calibration (such as pH meters, O_2 Saturometers for water sampling), or use of commercial laboratory field test kits with the manufacturer's recommended QA/QC for onsite data collection.

B. Laboratory Activities Review: the review of laboratory activities shall be conducted by the QAC or by one or more persons knowledgeable in the activities being reviewed including:

1. Completeness of laboratory records: this review determines that incoming samples have been processed and documented according to accepted protocols (Appendix D). The review shall include an audit of completed records of all testing analysis and associated QC samples.

2. Evaluation of Sample Management: this review identifies that proper procedures were used in receipt, handling and storage of incoming samples.

3. Evaluation of General Laboratory Techniques: this review identifies that laboratory operations not addressed by accepted protocols are conducted using acceptable methods. This includes but is not limited to glassware cleaning procedures, use of analytical balances, pipetting techniques, use of sonicators, water baths, and general laboratory operations. . Other objectives of this review are to identify proper use and maintenance of laboratory apparatus. This shall include but not be limited to:

   a. determining that the appropriate apparatus is used for the targeted analytical assay and that instrument location is suitable.

   b. determining that equipment is adequate and meets the needs for the capacity of average number of samples analyzed.

   c. determining that analytical equipment and other apparatus used is periodically cleaned, inspected, calibrated and serviced as deemed necessary (Appendix E).

4. Evaluation of Reagent and Standards Preparation: this review addresses whether all reagents and standards meet the specifications of accepted protocols (Appendix D). The

review also addresses whether preparation and dilutions of these reagents/standards are properly recorded, appropriately labeled and stored in suitable containers under recommended storage conditions.

5. Evaluation of analytical data with respect to method detection limits (MDL).

   a. ELISA – when the established protocols are employed, all data points derived from positive control titration replicates, as well as substrate negative controls are analyzed against established parameters to determine that the assay equipment is performing appropriately during every ELISA run.

A. Laboratory Performance Review: An internal laboratory performance review can be conducted as deemed appropriate or necessary. This review will evaluate the "performance" of the standard protocol of an individual assay through analysis of unknown samples or reference samples (samples containing a known target pathogen) submitted by the internal laboratory auditor (i.e. QAC). These samples can be submitted as blind samples from an independent outside source. The result of this audit provides documentation of bias of the analytical process for that individual assay for the target pathogen.

IV. **Objectives: (External Audit Plan)**

The external audit team should consist of at least three members external to the FHL to be reviewed, with knowledge of the activities to be reviewed. Since the purpose of external audits is to identify problems overlooked or undetected by the internal reviews, objectives of the external auditing committee shall include but not be limited to all objectives listed under the Internal Auditing Plan.

V. **Proficiency and Ring Testing Programs**

A. Proficiency and ring testing programs can be implemented by individual FHLs with the desire to evaluate their proficiency in performing standard protocol of a particular assay. It involves the analyses of sets of blind samples (infection status of tissues known only to the supplier) submitted to the FHL by an outside source. Participation in a ring testing program would allow the FHL to measure their level of proficiency against other FHLs performing testing on identical blind sample sets. Individual FHLs can arrange with commercial or research facilities to provide blind samples for proficiency testing, or can coordinate with other FHL's to accomplish multi-laboratory ring testing as well. Proficiency testing can also be achieved through contract of commercial labs which provide such services (see http://www.phppo.cdc.gov/mlp/EQA_qlist.asp).

B. Samples for a proficiency testing program would be processed according to the appropriate protocols by the FHL within a given time frame, and results reported to a reporting individual for analysis.

1 Evaluation of assay performance problems should involve, at minimum, the determination of the following:

a. reagent quality (storage, preparation, shelf-life)
b. quality, source and performance of standards and controls
c. equipment calibration and maintenance
d. equipment performance
e. personnel training and performance
f. sample integrity (storage, processing, etc.)

APPENDIX B-1
FORMS AND DOCUMENTATION

Suggested Documentation

Documentation Files

- ✓ Technical Qualifications File – contains resume of qualifications, skills, experience and references to training received and conferences attended for each current staff member.
- ✓ Equipment Manuals
- ✓ Equipment maintenance and calibration/certification (2 year retention)
- ✓ Reagent and materials source information
- ✓ Material Safety Data Sheets
- ✓ Safety Audits
- ✓ QA/QC Audits
- ✓ Proficiency testing results
- ✓ QA/QC Quarterly meeting minutes
- ✓ Case Histories (7 year retention)
- ✓ Case History Reports (7 year retention)
- ✓ General communications relating to Case Histories

Documents

- ✓ AFS-FHS (American Fisheries Society-Fish Health Section). 2004. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2004 edition. AFS-FHS, Bethesda, Maryland.
- ✓ OIE Manual of Diagnostic Tests for Aquatic Animal Diseases, 4th Edition. 2003. Office International Des Epizooties. Paris, France. (http://www.oie.int)
- ✓ Regional Fish Health Policies
- ✓ State/Provincial Fish Health Laws and policies
- ✓ Station Safety Plan
- ✓ Hazard Communication Plan
- ✓ Chemical Hygiene Plan

Logs to Maintain

- ✓ Equipment Maintenance
- ✓ Separate activity logs for virology, bacteriology and other laboratories (this will include documentation of protocol deviation and justification, as approved by FHL Director)

Computer files to Maintain

- ✓ Case History Database
- ✓ Reagent and media inventory

- ✓ Supply inventory

APPENDIX B-2
FORMS AND DOCUMENTATION

Audit - Field and Sampling Checklist

Quality Control Item or Procedure Noted	Suitable √	Not Suitable (Explain)
Samples are collected by, or under supervision of AFS/FHC Fish Health Inspector or veterinarian.		
Sterile utensils, loops, containers, tubes, vials used where appropriate.		
Samples labeled appropriately.		
Proper use of disinfectants.		
Proper means of disposal of contaminated waste.		
Proper means of specimen collection including humane methods for animal handling		
Sample vessels are appropriately labeled.		
Sample information recorded for case information.		
Specimens, tissues, and media are kept cool where appropriate throughout necropsy and transfer to laboratory.		
Tissue samples are obtained in a manner which avoids contamination.		
Cross contamination between samples prevented.		
Cross contamination between lots prevented.		
Sample transfer from field to lab – samples packaged to avoid leakage and cross-contamination during transfer.		

Attach notes and documentation of audit.

APPENDIX B-3
FORMS AND DOCUMENTATION

Audit - Laboratory Documentation/Sample Tracking Checklist

Documentation	Suitable √	Not Suitable (Explain)
Approved Laboratory and Diagnostic protocols available to all staff.		
Case History information entered onto CHR appropriately.		
Case History Record form printout available with appropriate information and attachments for each case.		
All racks and/or vessels containing processed samples are labeled with CHR number – all tubes with sample/lot I.D.		
Sample logs maintained in individual laboratories (bact., vir., etc) for each case.		
All data generated from all sample assays documented, in ink, on sample data forms.		
Results properly reported and dated on CHR, with appropriate sample data forms attached.		
Technical Qualifications File on all personnel.		
Appropriate equipment labeled with date of last calibration/certification.		
Equipment maintenance log with minimum of 2 years record of certification/calibration:		
Laminar flow hoods		
Fume hoods		
Balances		
Micro-pipetters		
Spectrophotometers		
Thermocyclers		
Thermometers		
Meters (pH, D.O., etc)		
CHR maintained in FHL files for minimum of 7 years.		
Material Safety Data sheets readily available for all materials.		
All reagent containers properly labeled.		

APPENDIX B-4
FORMS AND DOCUMENTATION

Audit - Laboratory and Assay Performance Checklist
BACTERIOLOGY

Quality Control Item or Procedure	Suitable √	Not Suitable (Explain)
Media Preparation		
Gram staining procedures		
Biochemical procedures and systems (API/other)		
Use and maintenance of control isolates		
Antibiotic sensitivity procedures		
Confirmatory Procedures		
Fluorescent Antibody Procedures:		
Sample preparation, fixation and storage		
Conjugate preparation and filter sterilization		
Staining procedures		
Positive/negative controls		
Slide examination and quality of preparation		
Proper disposal of wastes		
Overall aseptic technique, disinfection and sterilization procedures/equipment		
Level II Lab containment standards and building hygiene		

APPENDIX B -5
FORMS AND DOCUMENTATION

Laboratory and Assay Performance Checklist

VIROLOGY

Quality Control Item or Procedure	Suitable √	Not Suitable (Explain)
Maintenance of stock cell lines-passage if confluent cell monolayers		
Semi-annual mycoplasma screening and virus susceptibility checks of fish cell lines		
Media preparation and contamination checks		
Glassware cleaning procedures to ensure cell health		
Optimum cell lines, incubation temperatures and times used		
Centrifugation techniques		
Viral plate reinoculation procedures		
CPE examination frequency/virus identification		
Virus confirmation procedures		
Proper disposal of wastes		
Overall aseptic technique, disinfection and sterilization procedures/equipment		
Level II Lab containment standards and building hygiene		

PARASITOLOGY – Refer to Parasitology Protocols

Quality Control Item or Procedure	Suitable √	Not Suitable (Explain)
Sample storage		
Pepsin/trypsin digest procedure		
Positive controls		
Disinfection and cleaning between samples		
Confirmation procedures (PCR or histology)		
Proper sample archiving		
Slide preparation, fixation and storage		
Staining procedures		
Microscopic procedures		
Proper disposal of wastes		
Overall aseptic technique, disinfection and sterilization procedures/equipment		
Level II Lab containment standards and		

building hygiene		

APPENDIX B-6
FORMS AND DOCUMENTATION

Laboratory and Assay Performance Checklist
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Quality Control Item or Procedure	Suitable √	Not Suitable (Explain)
Reagent preparation and storage		
Quality of glassware (acid washed, dedicated)		
Sample preparation		
Utilization of positive/negative controls		
ELISA plate – use of appropriate standard controls and replicates		
Adherence to protocol		
Interpretation of results		
Confirmation		
Proper disposal of wastes		
Overall aseptic technique, disinfection and sterilization procedures/equipment		

Protocol – Proper procedure can be referenced in appropriate USFWS Operational Handbook protocols.

APPENDIX B-7
FORMS AND DOCUMENTATION

Laboratory and Assay Performance Checklist
POLYMERASE CHAIN REACTION (PCR)

Quality Control Item or Procedure	Suitable √	Not Suitable (Explain)
Reagent preparation and storage		
Dedicated pipetters		
Aerosol barrier and/or positive displacement pipette tips utilized.		
Latex or nitrile gloves utilized and changed appropriately		
Appropriate laboratory set-up		
Sample handling/preparation		
DNA/RNA Extraction procedures		
Proper controls		
Quantification of template		
Master mix preparation: documentation of reagent lots and concentration		
Sample loading and controls		
Proper handling of tubes between first and second rounds in nested PCR		
Proper centrifugation		
Thermocycler properly programmed		
Gel lane documentation and loading		
Gel photodocumentation		
Interpretation of results		
Proper storage and documentation of samples, templates and PCR products		
Proper disposal of wastes		
Overall aseptic technique, disinfection and sterilization procedures/equipment		

APPENDIX C
North American Reference Laboratories

The following chart contains facilities that can be contacted for information, expertise and services listed:

Facility Information	Contact Information	Services/Expertise
American Type Culture Collection (ATCC) P.O. Box 1549 Manassas, VA 20110-2209	[To be named] Phone 703-365-2718 Fax 703-365-2730 http://www.atcc.org	Source for pathogen control isolates, cell culture stocks, reagent controls
Atlantic Veterinary College Aquatic Diagnostic Services 550 University Ave. Charlottetown, Prince Edward Island, Canada. C1A 4P3	Dr. David Groman Phone 902-566-0864 FAX 902-566-0723 aquaticdx@upei.ca	Salmonid and other finfish diseases
Auburn - Dept. of Fisheries and Allied Aquacultures Auburn University Auburn, AL 36849	John Grizzle, Ph.D. Phone 334-844-3474 FAX 334-844-9208 jgrizzle@acesag.auburn.edu	Largemouth Bass Virus
Cornell University -Aquatic Animal Health Program Dept. Microbiology and Immunology College of Veterinary Medicine Cornell University Ithaca, NY 14853	Paul R. Bowser, Ph.D Phone 607-253-3365 FAX 607-253-3384 prb4@cornell.edu Internet: http://web.vet.cornell.edu/Public/FishDisease/AquaticProg/	Finfish diseases
Department of Fisheries and Oceans Canada Fish Health and Parasitology Section 3190 Hammond Bay Road Nanaimo, British Columbia Canada V9T 6N7	Dr. Susan Bower (Shellfish Health Unit) Phone 250-756-7077 bowers@dfo-mpo.gc.ca FAX 250-756-7053 Dorothee Kieser (Fish Pathology Program) Phone 250-756-7069 Fax 250-756-7069 kieserd@dfo-mpo.gc.ca	Shellfish Diseases OIE Designated Reference Lab Salmonid and other finfish diseases; Approved lab under the Canadian Fish Health Protection Regulations
Department of Fisheries and Oceans Canada Fish Health unit 501 University Crescent Winnipeg, Manitoba	Brian Souter Phone 204-983-5125 FAX 204-984-2402 souterb@dfo-mpo.gc.ca	Freshwater finfish viral & parasite diseases

Canada R3T 2N6		

APPENDIX C -2
North American Reference Laboratories

Facility Information	Contact Information	Services/Expertise
Department of Fisheries and Oceans Canada Gulf Fisheries Center, Fish Health Unit P.O. Box 5030 (343 University Ave) Moncton, New Brunswick Canada E1C 9B6	Anne-Margaret MacKinnon Fish Health Section Phone 506-851-6081 mackinnona@mar.dfo-mpo.gc.ca Mary Stephenson (Shellfish Health Unit) Phone 506-851-6983 stephensonm@dfo-mpo.gc.ca FAX 506-851-2079	Salmonid and other finfish diseases; Molecular diagnostics Shellfish Diseases
Hawaii Institute of Marine Biology, School of Ocean and Earth Sciences Technology University of Hawaii, P.O. Box 1346, Kaneohe, Hawaii 96744,	Dr J.-A. Leong Phone: 808-236-7401 Fax: 808-236-7443 joannleo@hawaii.edu	OIE Reference Laboratory for IHN
Micro Technologies, Inc. 41 Main St. Richmond, ME 04357	Deborah Bouchard Phone 207-737-2637 FAX 207-737-4504 dbouchard@microtechnologies.biz	USDA Approved; Diagnostic and PCR Services; Shellfish and Finfish Inspection Services
Mississippi State Fish Diagnostic Laboratory College of Veterinary Medicine, Mississippi State University Box 9825 Spring Street, Mississippi 39762	Dr L.A. Hanson Phone: 662-325-1202 Fax: 662-325-1031 hanson@cvm.msstate.edu	OIE Reference Laboratory for Channel catfish virus disease and Enteric septicemia of catfish (*Edwardsiella ictaluri*)
Oregon State Center for Fish Diseases Research, Department of Microbiology 220 Nash Hall Oregon State University Corvallis, Oregon 97331-3804	Dr M. Kent Phone: 541-737-4441 Fax: 541-737-0496 michael.kent@orst.edu	OIE Reference laboratory for Piscirickettsiosis (*Piscirickettsia salmonis*)
Pisces Molecular, LLC 2200 Central Avenue, Suite F Boulder, CO 80301	John Wood, PhD Phone 303-546-9300 Fax 303-546-9400 jwood@pisces-molecular.com	Molecular diagnostic services, PCR assay research & development, strain genetic identification & analysis.
USDA Agriculture Research Service HKD Stuttgart National Aquaculture Research Center P.O. Box 1050 Stuttgart, AR 72160	Andrew J. Mitchell, Ph.D. Phone 870-673-4483 FAX 870-672-7710 dmitchell@spa.ars.usda.gov	Fish Disease Research
U.S. Fish and Wildlife Service Fish Health Centers Nine centers located throughout the USA	USFWS Fish Health Center locations can be obtained on the internet at: http://fisheries.fws.gov/FHC/FHCcu.htm	General and regional fish disease information

APPENDIX C-3
North American Reference Laboratories

Facility Information	Contact Information	Services/Expertise
U.S. Geological Survey National Fish Health Research Laboratory 11700 Leetown Rd. Kearneysville, WV 25430	Frank Panek Phone 304-724-4430 FAX 304-724-4435 frank_panek@usgs.gov	Fish Disease Research
U.S. Geological Survey Western Fisheries Research Center 6505 N.E. 65th St. Seattle, WA 98115	Dr. James Winton Phone 206-526-6282 Fax 206-526-5564 jim_winton@usgs.gov	Fish Disease Research; OIE Reference Laboratory for IHN and BKD
UC-Davis, Department of Medicine School of Veterinary Science University of California Davis, CA 95616	Dr. Ron Hedrick Phone 916-752-3411 rphedrick@ucdavis.edu	Fish Disease Research;
University of Maryland VA-MD Regional College of Veterinary Medicine Aquatic Animal Health Center 8075 Greenmead Drive College Park, MD 20742-3711	Ana M. Baya Phone 301-314 6837 FAX 301-935-6072 ambaya@wam.umd.edu	USDA Approved; Diagnostic Services
VA Tech - Dept. Biomedical Sciences and Pathobiology VA-MD Regional College of Veterinary Medicine Virginia Polytechnic Institute and State University Phase III, Duck Pond Dr. Blacksburg, VA 24061	Stephen A. Smith, D.V.M., Ph.D. Phone 540-231-5131 FAX 540-231-6033	Fish Disease Research; Diagnostic Services
Washington Animal Disease Diagnostic Laboratory College of Veterinary Medicine Washington State University PO Box 647034 Pullman, WA 99164-7034	Dr. Kevin Snekvik Phone 509-335-9696 FAX 509-335-7424 ksnek@vetmed.wsu.edu waddl@vetmed.wsu.edu Internet: http://www.vetmed.wsu.edu/depts_waddl/aquatic.asp	USDA Approved; Inspection and Diagnostic Services; Molecular diagnostics
Office International des Epizooties (OIE) Aquatic Animals Commission **Note: OIE Reference Labs must be reported to upon initial confirmation of an exotic pathogen in any species of fish in the US or diseases in commercially reared fish involved in international trade.**	OIE Reference Laboratories and designated Experts for aquatic animal diseases on the Internet: http://www.oie.int/fdc/eng/Diseases/en_reflabslist.htm Email: rr.americas@oie.int or fdc@oie.int	Lists all OIE reference labs and expertise in aquatic animal diseases located throughout the world.

APPENDIX D
SOURCES FOR FISH HEALTH ASSAY PROTOCOLS

American Fisheries Society, Fish Health Section. 2002. Suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 5th edition. American Fisheries Society, Fish Health Section, Bethesda, Maryland.

OIE Manual of Diagnostic Tests for Aquatic Animal Diseases, 4th Edition. 2003. Office International Des Epizooties. Paris, France. (http://www.oie.int)

Handbook of the U.S. Fish & Wildlife Service Aquatic Animal Health Procedures and Protocols. 2002. USFWS, National Fish Hatchery System, Arlington, VA (http://policy.fws.gov/fh_handbook/toc.htm)

50 CFR Chapter 1, Part 16, Code of Federal Regulations, U.S. Government Printing Office, Washington D.C. 2003. (http://www.access.gpo.gov/nara/cfr/cfr-table-search.html)

Fish Health Protection Regulations Manual of Compliance. Department of Fisheries and Oceans.1984.Fish Health Protection Regulations: manual of compliance.Fish. Mar. Serv. Misc. Spec. Publ. 31 (Revised): 32 p. (http://www.dfo-mpo.gc.ca/science/aquaculture/aquaculture_e.htm)

APPENDIX E
EQUIPMENT CALIBRATION/MAINTENANCE

The following chart provides a guide for calibration of standard equipment used in the Fish Health Laboratory. All Intervals suggested are subject to individual manufacturer and/or equipment manual.

EQUIPMENT	EXTERNAL CALIBRATION INTERVAL	INTERNAL CHECK INTERVAL	PARAMETERS TO CHECK	INSTRUMENTS REQUIRED
Autoclaves		Daily	Temperature & pressure sustained during operation	Pressure gauge, safety valve, temperature gauge, indicator strips
Balances	3 years	Weekly	Linearity, zero point, accuracy, level	Calibration reference weights
Biosafety Cabinets/hood	1 year	Weekly	Air flow, UV bulbs	Anemometer, vacuum meter
Centrifuges		During operation	Balance, speed, temperature, timer	Manufacturer provided
Electrophoresis Units		Daily / as used	Structural integrity, voltage	Volt meter (power unit)
ELISA readers	1 year	Monthly	Lamp stability, optics, filters	Calibration plate
Freezers		Daily	Visual, thermal stability	Calibrated thermometer or pyroprobe
Incubators		Daily	Temperature	Calibrated thermometer
Micro-pipettes	3 years	Daily / as used	Dirt and damage, volume delivery accurate	70% ethanol. If damaged, send for repair, graduated tips
		Every three months	Volume accuracy	Gravimetric method
Micro-pipettor (automatic)		Every three months	Volume, accuracy	Manufacturer's procedure
Microscopes	3 years (cleaned)	Daily / as used	Alignment, bulbs	Manufacturer procedure
pH meters		Daily / as used	Electrode drift or reduced response	Check against two buffer solutions
Plate washer (automatic)		Daily / as used	Nozzles, hoses, vacuum, pH of wash buffer	Distilled water for flushing, instrument gauges, pH meter
Thermal-cyclers	If needed	Annually	Block Temperature	Probe or check with sample replicate matrix
Thermometers (digital)	1 year	6 months	Check at point in working range against reference thermometer	Certified reference thermometer
Thermometers (liquid in glass)	10 Years	6 months	Check at point in working range against reference thermometer	Certified reference thermometer
Thermometers (reference)	10 years	Before use	Check at ice point	
Timers		2 years	Accuracy	
Water baths		Daily / as used	Temperature and correlation with controls	Calibrated thermometer
Water Purification (deionizer)		Daily / as used	Conductivity and meter battery	Conductivity meter, voltmeter
Water purification (glass distillation)		Weekly / as used	Conductivity, pH, hardness	Conductivity and pH meter, hardness test kit

Appendix F
Disinfectant Solution Guidelines

1) Alcohols – 70% isopropanol or ethanol; general skin and surface disinfectant
   NOTE: Ineffective against bacterial spores; evaporates rapidly; flammable; can damage rubber, plastic and flooring surfaces.

2) Iodophors - A broad spectrum disinfectant for hands (available in soap form), lab surfaces, and instrument disinfection according to the following:
   100 ppm free iodine solution – exposure for 10 minutes
   200 ppm free iodine solution – exposure for 1 minute
   25 ppm free iodine solution – exposure for 3 hours
   NOTE: "Proper dilution to 1% iodine is necessary for maximum killing effect and minimal toxicity. More concentrated solutions are actually less efficacious, presumably due to stronger complexation preventing free iodine release. It takes approximately 2 minutes of contact time for release of free iodine (Lavelle et al. 1975). Literature reports indicate that iodophors are quickly bactericidal, virucidal, and mycobactericidal but may require prolonged contact times to kill certain fungi and bacterial spores…Iodophors formulated as antiseptics are not suitable as hard-surface disinfectants, due to insufficient concentrations of iodine…Iodophor solutions retain their activity in the presence of organic matter at pH <4…" (Veterinary Pharmacology and Therapeutics 7th ed., ed by H. Richard Adams.

3) Quaternary ammonium compounds – includes Roccal, Hyamin, and other brand name agents. Use for general laboratory surface and equipment disinfection and cleaning. Dilute according to manufacturer's instructions.
   NOTE: Ineffective against bacterial spores, some viruses, and mycobacteria

4) Sodium Hypochlorite – Broad spectrum disinfectant for use on waste liquids, instrument disinfection, surface decontamination and emergency spill decontamination. Use as follows:
   a. General disinfecting of bacterial and viral contamination
      i. 200 ppm active sodium hypochlorite solution – exposure for 1 hour minimum
      ii. 500 ppm active sodium hypochlorite solution – exposure for 10 minutes to 1 hour
   b. Disinfection of parasitology lab waste containing myxosporean contamination
      i. 5000 ppm active sodium hypochlorite solution - exposure for 10 minutes minimum.
   c. Solution preparation
      i. Concentrations of active sodium hypochlorite contained within household bleach vary with brand used. It is recommended that solutions be prepared according to the following calculation:

$$\frac{(\text{ppm active sodium hypochlorite desired})\ (\text{Gal of Water})\ (128)}{(\text{Percent of active sodium hypochlorite})\ (10{,}000)} = \text{ounces of household bleach product for solution}$$

d. **NOTE:** Toxic to skin; corrosive to metals; inactivated by organic matter; deteriorated over time – shelf life of solutions exposed to air and light is less than one week.

5) Neutralization – chlorine and iodine are toxic to living organisms, so neutralization may be necessary prior to disposal. Using a 1% solution of sodium thiosulfate, the following volume can be used to neutralize solutions as follows:

Sodium hypochlorite: 28.5 mL (number of liters of disinfecting solution X concentration ppm)/100

Iodophor: 7.8 mL (number of liters of disinfecting solution X concentration ppm)/100

(Info derived from Meyer, et al, 1983; Piper, ed. 1983; and on the web – http://www.mcgill.ca/eso/newweb/lab/biosafe.htm)

See also : OIE *Manual of Diagnostic Tests for Aquatic Animal Diseases*, 4th Edition. 2003. Office International Des Epizooties. Paris, France. (http://www.oie.int) Section 1.1.5

APPENDIX G

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