

# QUALITY ASSURANCE/ QUALITY CONTROL

## For the U.S. FISH AND WILDLIFE SERVICE FISH HEALTH LABORATORIES

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## **I . Introduction**

The purpose of the QA/QC Program is to ensure the quality of the information and results generated by US Fish and Wildlife Service (USFWS) fish health centers (FHC) for fish health inspection and diagnostic assays. The personnel of each laboratory are to be familiar with the QA/QC Program described in this document, and implement its policies and procedures at all times.

Full implementation of this program will necessitate coordination on a national basis. The responsibilities of a National Fish Health Center Quality Improvement Coordinator (NQIC) would include coordination of external audits and proficiency testing programs, analysis and reporting of audit results, assessment of corrective actions, assay validation studies, and would provide guidance for internal laboratory performance audits. The NQIC would play a key role in full and consistent implementation of this program at USFWS FHCs throughout the U.S. The achievement of this program will not only promote the credibility of the USFWS Fish Health Program, but will serve as a model for other (non-USFWS) fish health labs as they strive for quality improvement.

## **II. Policy and Implementation**

### **A. Management Responsibilities**

1. The FHC Director shall have overall responsibility for the technical integrity of the tests as well as for interpreting, analyzing, documenting and reporting results. 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 affect the integrity of the tests are corrected and documented, and;
  - c. All test data are accurately and precisely recorded and reported.
2. The FHC Director shall be accountable for QA/QC concerns of the FHC to the appropriate Regional Directorate.

### **B. Personnel/ Personnel Responsibilities**

1. Each FHC 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. All employees will be encouraged to discuss concerns with the Quality Assurance Coordinator for the FHC and the NQIC.

2. Each employee shall follow protocols described within the USFWS Handbook for each test conducted. Deviations from these protocols shall be approved in advance by the FHC Director and the reasons for the deviation shall be documented.
3. 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.
4. Each employee shall take the necessary precautions to avoid contamination of the test, control, and reference substances.
5. 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. One employee at each FHC is designated as the Quality Assurance Coordinator (QAC). The QAC should have responsibility and authority to implement this program. QAC responsibilities include:
  - a. Documenting and communicating to the FHC Director and the NQIC any deficiencies regarding proper implementation and monitoring of this QA/QC Program
  - b. Drafting corrective procedures, alternatives or improvements in this program for approval by the FHC Director and the NQIC.
  - c. Implementing all necessary quality controls to ensure the accuracy and precision of reported data.
  - d. Monitoring laboratory practices to verify continuing compliance with policies and procedures.
  - e. Evaluating instrument calibration and maintenance records.
  - f. Ensuring the validation of new technical procedures, in cooperation with the NQIC.
  - g. With the FHC Director and NQIC, investigate technical problems, proposing remedial actions, and verifying their implementation.
  - h. Providing recommendations for training to improve the quality of laboratory staff.
  - i. With the FHC Director, cooperate with and accompany teams conducting external QA/QC audits of the facility (See Appendix A).
  - j. Ensure that the FHC participates in all proficiency and “ring” testing programs available.
  - k. Hold a quarterly QA/QC meeting with staff members, and maintain records of minutes taken at these meetings.

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, and experience.
    - b. References to all training classes, seminars, short courses, and conferences attended.
  3. A checklist of recommended documents and files to be maintained at each FHC is provided in Appendix C.
- D. Laboratory Facilities
1. Sample Collection/Necropsy: All samples will be collected by staff members of the regional USFWS FHC, or an individual specifically trained and delegated to do so, under supervision or approval of fish health personnel. Necropsy and sample collection is performed in accordance with protocols established in the USFWS Handbook.
  2. Sample Processing: Virology, parasitology, microbiology, and molecular techniques will be performed within a USFWS FHC laboratory facility. Those laboratories possessing qualified staff and facilities will also perform histopathological assays. Center Labs which do not have histology capabilities ship necessary samples for analysis to an approved academic or government laboratory with expertise in fish Histopathology .
  3. Laboratory Space and Design
    - a. USFWS FHC labs comply with national and local standards of health and safety.
    - b. Each FHC is separated into specific areas for administrative activities, fish handling, and laboratory testing for all procedures performed by the FHC.
    - c. Each laboratory room is equipped with adequate space and environmental conditions to properly perform assigned tasks.
    - d. Desk space is provided for each employee along with file cabinets and book shelves to store records, reference texts and other documents.
    - e. Storage spaces are adequate to maintain equipment, supplies, samples, and chemicals without danger of cross contamination.
- E. Laboratory Equipment and Supplies
1. Equipment - The Quality Assurance Coordinator (QAC), or designated staff of each Center is responsible for maintenance of all laboratory equipment, and recording all maintenance records in a common log (See Appendix D for suggested forms and information):

- a. The QAC ensures that all microscopes, balances, pipettors, 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.
  - b. Where appropriate, equipment maintenance and temperature information is posted on its surface (hoods, balances, refrigerator/freezers, incubators).
  - c. Defective or suspect equipment is taken out of USFWS use until repaired, tested and recalibrated.
  - d. Microscopes, hoods and Equipment used for generating measurements are cleaned, calibrated and standardized professionally at recommended intervals (Appendix D).
  - e. Each appropriate item of equipment possesses an inventory number for identification. Records of calibration and maintenance documentation are kept for each instrument and microscope.
2. Reference stocks and reagents - The QAC is responsible 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 be labeled by name, 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 (<http://www.nfpa.org>).
  - b. Reagent bottles are labeled to identify contents, titer or concentration, storage requirement, expiration date and safe use and storage requirements (NFPA labels).
  - c. Reference stocks and reagents are handled in a manner which precludes the possibility of contamination, deterioration, or damage to the substance.
  - d. All reagents, serums, cell lines, and laboratory supplies are of high quality and purchased through a reputable supplier (a reputable supplier is one that employs appropriate quality assurance standards in the manufacturing of materials for clinical use). To ensure quality performance during all laboratory assays, specific product and manufacturer sources are provided in the USFWS Handbook where deemed necessary.
  - 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. All FHCs are required to have a “Safety and Chemical Hygiene Plan” approved by respective Regional Safety Officers. All laboratory personnel are required to utilize equipment and reagents in compliance with that Center’s plan.
  - h. Mixture of substances - When test, control, or reference substances are mixed, the date of preparation, initials of preparer, 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 each FHC laboratory, and details of all cases are maintained in electronic database format.
    - a. The case history number uniquely identifies the test samples on receipt and tracks the case throughout the laboratory. Upon receipt the case history number is assigned to sufficiently identify all sample containers and racks used in the processing of the samples.
    - 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
      - 5) Name of Sampler
      - 6) Recorder Initials
      - 7) Species and Age-class of fish
      - 8) Lot designation number (hatchery records)
      - 9) Condition of Samples at receipt if notably compromised
      - 10) This Case History Record (CHR) cover sheet also identifies specific numbers and tissue materials sampled for the following lab assays: Bacteriology, virology, parasitology, Serology, Histology and Other. In addition, any descriptive information received with the samples is attached to the CHR.
    - c. All tubes, bags, or other sample containers are sufficiently labeled to allow for accurate tracking of samples through each laboratory area.
    - d. All CHR’s are transcribed in black 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 track the samples received into the area, assays requested and performed and results obtained. At completion of all assays, the results are attached to and/or recorded onto the original CHR and filed in the appropriate area for cross-reference.
    - c. When all assays are completed and results are obtained, the CHR with all necessary attachments is provided to the designated biologist for a Case Report write-up. All reports refer to the appropriate CHR number, and copies are maintained in FHC files.
  3. Record Retention
    - a. Hard copies of records are retained in FHC 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 FHC follows specific protocols as described in the USFWS Handbook, depending upon the type of case being conducted. As already required, any deviations will be documented and kept on file for inspection. All protocol deviations must be documented with a description of the procedures used, and/or citation from the literature.
    - a. Fish Health Inspection samples are assayed according to the USFWS Inspection Handbook, and/or other state regulation, geographical fish health compact guidelines, or international requirements which may apply.
  - ~~2.~~ The National Wild Fish Health Survey (NWFHS) samples are assayed according to protocols described in the NWFHS Laboratory Procedures Handbook. Some assays performed for diagnostic cases may utilize protocols which are not available in the USFWS Handbook. Center Directors are responsible for approving such protocols prior to their use
  3. 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 or recorded onto 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, as necessary according to individual protocols. Standards and controls are kept separate from samples when stored. Detailed information on controls, reagents and media used for each test is described in the USFWS Handbook.
    - 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 shipping/transport container holding the samples is externally decontaminated prior to opening. Upon opening of container, the inside of the container is inspected for sample spillage. All shipping containers should be disinfected properly following use.
      - 3) Tubes, bags or other items containing tissue samples are removed, externally disinfected, 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 lab coats within laboratory (coats are laundered regularly).
      - 2) Wash hands with antibacterial soap before and after lab.
      - 3) Clean countertops with an appropriate disinfectant preceding and following any lab work. (See Appendix E for a list of appropriate disinfectant solutions.)

- 4) Decontaminate container surfaces with alcohol spray 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 when spills occur.
  - 7) Decontaminate waste materials from sample processing/ necropsies in an appropriate disinfectant solution (Appendix E) prior to disposal, freeze excess necropsy material in plastic bags, or autoclave prior to proper disposal. If autoclaved, use autoclave tape for assurance of proper sterilization.
  - 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 the Fish Health Center laboratories. The following are general considerations for maintaining QA/QC of samples obtained during a lab necropsy:
- 1) Specimens, tissues and media are kept cool where appropriate throughout necropsy.
  - 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 Handbook protocols
  - 5) Properly dispose of all waste materials immediately following necropsy.
  - 6) Disinfect all work areas, and appropriate equipment involved in examination of tissues during necropsy.
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 they are

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 bacteriology 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°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°C for 15 minutes (Turner, et al 1999). (When autoclaving large volumes of lab waste, time may need to be increased to insure the core temperature of waste reaches 121°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 chlorine solution for at least 10 minutes (Appendix E)
  - 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.
  - 3) Cover work areas with bench paper or aluminum foil 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 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 of samples, the date sample was taken in the field. The log must contain designated spaces for logging each case out as they are 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 document at a minimum the CHR number, each sample identification number as well as description and results of all biochemical, morphological, and serological test conducted.
- 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 bacteriology lab:

- 1) Dispose of used media (Petri plates and tubes) by autoclaving at 121°C for 20 minutes. Media can then be safely discarded with the trash.
- 2) Glassware tops should be inspected and loosely secured prior to proper sterilization. Foil sheets to be used should be checked for pin-holes and tears, and proper fit must be ensured.
- 3) Soak depression glass slides used for hanging drop motility assays in chlorine solution (Appendix E) prior to cleaning with laboratory grade detergent.
- 4) Soak used disposable loops, pipettes and other expendable items which become contaminated in chlorine solution before disposing of in the trash. Alternately, these items can be disposed of in bio-hazard bags and autoclaved.

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. The following includes materials and handling tips to ensure this goal is met:

- 1) Loops: Use sterile disposable loops whenever possible. Remove loops from packaging just prior to use. Place in chlorine solution (Appendix E) 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 media it is being transferred onto. If this should happen, dispose of the loop and get a new one to complete the transfer. Metal loops should be incinerated between transfers.
  - 2) Avoid talking, coughing and sneezing when bacterial cultures and media are exposed to the air.
  - 3) Petri Plates: Prior to use, media 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 left up 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.
- d. Cross-Contamination Control - In addition to the measures listed above, the following guidelines are followed to avoid cross contamination of organisms between bacteriological case histories:
- 1) Each work station should contain only one case history at a time.
  - 2) Work stations should be decontaminated thoroughly between cases.
  - 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 properly identified with the CHR number.
- e. Media and Reagent Preparation:
- 1) Prior to media preparation under the laminar flow hood, spray surface with isopropyl alcohol and allow surfaces to be exposed to UV light for approximately 10 minutes prior to use. Allow the blower to run in the

hood for approximately ten minutes prior to use as well.

- 2) Plated media which requires exposure while drying 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 autoclaved media and reagents is performed under a decontaminated, laminar flow hood. Media which is distributed 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 B.
  - 6) All new batches of serological reagents are tested using both positive and negative controls.
  - 7) All serological testing on sample material must have concurrent controls using known positive and negative material (cultures or tissues).
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 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 data logging system upon receipt in virology lab. The log is designed to record the number and type of samples and the date sample was taken in the field. The log must contain designated spaces for logging each case out as they are 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 at least twice per week, and on days when cases

are to close. Record and initial observations in virology log and on a data entry form. Indicate cell condition, presence or absence of contamination, toxicity or any apparent viral cytopathological effects (CPE), and condition of controls, including progression of CPE in virus positive controls.

- 5) 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.

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. In addition to those listed in Section II.H.2.b., the following steps will be taken to insure both aseptic technique and contaminant control within the Virology Laboratory:

- 1) When working in laminar flow hood allow blower fan and UV lamp to operate at least 10 minutes prior to working under the hood. Spray entire work surface and any materials to be used with disinfectant solution. Use of UV light after use, is recommended for additional protection.
- 2) Glassware stored in virology lab first must have been rinsed thoroughly with tap water, rinsed three times with tissue culture grade water, covered and autoclaved (the slightest residual detergents and reagents can adversely effect tissue culture). Glassware used for harsh chemicals (chlorine, acetone, HCl, etc.) should be labeled so 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, each media component should be checked separately to determine the source of contamination. Media should be filter sterilized using 0.2  $\mu\text{m}$  filter.
- 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 component and adding antibiotics to complete media. Fill out a “Record of Media Preparation” form for each lot of media prepared (Appendix C).
- 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 E for procedures and alternative disinfection).

c. Media

- 1) Upon receipt in virology lab, date all chemicals, reagents and media components. Whenever media, reagent or antisera are prepared label as to date of preparation and content.
  - 2) Each bottle, tube, flask or plate in virology refrigerator and incubators must be properly labeled or will be discarded.
  - 3) An inventory log should be maintained primarily for much-used disposable supplies, but other supplies such as media components, HBSS, and versene salts must also be monitored regularly.
- d. Cell Culture Stocks - Maintenance of healthy tissue cultures is imperative for all viral assays. The following are procedures routinely employed to ensure that healthy, sensitive cultures are used for each assay:
- 1) Prior to use, check culture for possible contamination, appearance of cell sheet, and pH of medium. If the medium is turbid, bacteria or yeast are probably present. Cotton-like tufts indicate fungal contamination. Contaminated cultures shall never be used for virology assays.
  - 2) Examine cells microscopically to make sure cells are healthy and the sheet is confluent.
  - 3) Allow recently thawed versene/trypsin solution (V/T) to warm to room temperature (15-20°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), media lot #, date.
  - 5) On an annual basis, cell lines for virology are sent to the appropriate reference lab for sensitivity testing and contamination checks. Cells of appropriate sensitivity to viral pathogens, which show no contamination are sub-cultured and distributed out to all USFWS Fish Health Center laboratories.
  - 6) Cell lines can be obtained from a variety sources listed in Appendix B.
6. Routine Quality Control for ELISA - The Enzyme-linked immuno-sorbant assay (ELISA) technique utilized by USFWS FHC laboratories was developed and standardized by researchers at the USGS Western Fisheries Research Center (Appendix B) for the detection of a specific protein produced by the bacterial fish pathogen, *Renibacterium salmoninarum* (Rs). 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. All other

ELISA systems or procedures utilized must be approved by the FHC Director and equipment operated according to manufacturer's guidelines.

- a. Sample collection and processing - follow USFWS Handbook protocols for collection and processing of samples in regard to QA/QC precautions.
- 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 particular wells on the 96 well ELISA plates prior to setting up the assay. These are recorded on the 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 the USFWS Handbook.
- 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 aliquots of small working volumes are prepared. Variations in activity may be due to several factors including freeze-thaw cycles and temperature fluctuations which may cause loss of activity when re-hydrated product is stored frozen in aliquots less than 50 uL.
- e. 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 give false-positive reactions in the ELISA. Properly decontaminated or sterile instruments are used for each sample collected to prevent cross-contamination between positive kidney tissues and negative samples. Keep samples cold and freeze as soon as possible. Standard procedures are followed in laboratory processing.
- f. Performing the assay: the assay is performed following the detailed protocol within the USFWS Handbook-National Wild Fish Health Survey Procedures unless the ELISA is used for hatchery stock – risk management purposes. . 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.

- g. 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 - KPL whole cell Rs preparation.
  - 2) Negative Control - Negative Kidney tissue, tested by ELISA and PCR (currently available from CA-NEV FHC, Appendix B).
  - 3) Blank - Wells receive PBS-T20 diluent only and serve as a Blank negative control. Conjugate Control - Wells receive Coating Solution without Coating Antibody (Cab) and serve as control for non-specific binding of the HRP-conjugated Ab to the well surfaces or the Coating Antibody.
  - 4) Substrate Control - Wells receive Milk Diluent without HRP-Ab and serve as the control for non-enzymatic production of the ABTS color reaction.
  - 5) 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.
- h. Positive threshold: for data interpretation, two standard deviations above the mean OD value of the Negative Control are used to establish the positive threshold. The Negative Control tissue consists of Chinook kidney tissue tested by ELISA and PCR and found to be negative for Rs 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.
  - 1) Example: the positive threshold is determined for an assay by calculating the mean and the standard deviation of Negative Control wells on all plates. Two standard deviations above the NC mean OD value is set for the determination of positive versus negative samples.
  - i. Data obtained from all control wells for each assay run is recorded in a common log book and reviewed monthly for data error trends. Data is analyzed for accuracy as described in Appendix A-section III. B. 7.
- 7. Routine Quality Control PCR - The Polymerase Chain Reaction<sup>1</sup> technique employs oligonucleotide primers to amplify base pair segments of genes specific for the target pathogen. Reverse Transcriptase-PCR (RT\_PCR) employs an initial

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<sup>1</sup> PCR is Patented by Hoffman-LaRoche, Inc.

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 the USFWS Handbook. 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 chlorine solution or appropriate DNA decontamination products to hydrolyze possible DNA contaminants. Likewise, tube racks should be soaked in chlorine for prior to use (Appendix E). Ultra violet decontamination is also recommended where possible.
  - 2) Store RNA and DNA samples and templates separately from PCR reagents and separately from controls.
  - 3) Employ aerosol resistant pipette tips and/or positive displacement pipettors during all extraction and amplification procedures. Separate pipettors 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 necessary to reduce the risk of contamination:
    - i. Master Mix (MM) area with UV hood - for mixing master mix reagents. Supply area with dedicated pipettors, ideally positive displacement pipettor/tips. No samples or amplified DNA shall be handled in or near this area.
    - ii. Sample Preparation Area - for loading of extracted (template) DNA from samples.
    - iii. Amplified DNA Area - supplied with pipettor dedicated for **amplified PCR product ONLY**. Handle any amplified PCR products in this area only, and clean area and equipment thoroughly after working with amplified DNA. Amplified DNA area should be equipped with a UV lamp for countertop 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 chlorine solution (Appendix E).

- 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 samples will effectively remove contaminants from previous samples.
  - 2) Keep samples cold and freeze as soon as possible at -70°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 buffer (RNA-Later<sup>®</sup> available from Ambion – cat. # 7020) can also be used and does not require that samples be frozen immediately.
- c. Extraction of DNA or RNA from samples - All general considerations should be employed including the following:
- 1) Use micro centrifuge tubes with locking lids so that contaminants do not escape during heating cycles that might cause breaching of tube cap 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 if available), 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. Although a successful reaction should yield approximately 100-300 ng DNA per µl, refer to individual protocols for guidance on amount of DNA or RNA recommended for each PCR reaction.
- 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 thermocycler, 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 cycle at 94°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) Photo-document all PCR gels and keep labeled photos attached to the appropriate CHR (or provide reference for finding the photo-document).
- 11) Archive properly documented PCR positive samples, templates, and/or amplified products at -80°C for future reference.

### 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 FHC maintains a Safety Manual and Chemical Hygiene Plan 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, 1988, U.S. Department of Health and Human Services, Public Health services, Center for Disease Control, and National Institutes of Health, 2nd edition, U.S. Government Printing Office, Washington D.C.

#### **IV. Review of the Guidelines**

These guidelines are to be reviewed and amended on an annual basis by the USFWS QA/QC Committee. Comments will be solicited in December of each calendar year from all fish health center personnel. Changes, and justification for no change, will be compiled and changes will be implemented. FHC Directors will perform a final review and adoption during the annual USFWS Fish Health Biologist meetings. Each current and new FHC employee must read and comply with the requirements of the quality assurance manual.

## **APPENDIX A**

### **Internal and External Audit Program**

#### **I. Introduction**

It is the goal of the USFWS FHC QA/QC program to assure that all data generated be scientifically valid, defensible and of known precision and accuracy. To assure that the obtained data meets these criteria both internal and external audits shall be periodically conducted under the direction of the laboratory's Quality Assurance Coordinator and the Center's 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 evaluation and establish the effectiveness of the FHC QA/QC program. 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 will require a periodic laboratory review by the Quality Assurance Coordinator or USFWS employee(s) assigned to this task to include:
  - 1. field and sampling activities review
  - 2. laboratory activities review
  - 3. laboratory performance review
  - 4. Results of the internal audit will be reported to the Center Director for assessment and correction of the observed deficiencies.
- B. The External Auditing Plan will require a periodic laboratory review by a qualified team of two USFWS FHC staff members, and two members external to the USFWS.
  - 1. The USFWS FHC staff members must not perform audits on their own lab of employment.
  - 2. The purpose of this assessment is to provide insight to QA/QC deficiencies not detected by the internal audit plan.
  - 3. The results of the external assessment along with necessary corrective actions are submitted to the Center Director for assessment.
- C. Check lists are provided (Appendix C-Forms) for guidance in performing these reviews.

#### **III. Internal Audit Plan**

- A. Field and Sampling Activities Review: the review of field and sampling activities shall be conducted by the QA Coordinator 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 the

USFWS Procedures Manual. 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 the procedures outlined in the USFWS Procedures Manual. This will include:
    - a. Reviewing that samples were collected under the direction of a fish health biologist of the USFWS.
    - b. Evaluating that appropriate tissue was collected for each sample assay with proper disinfection of tools or individual tools used between samples.
    - c. Evaluating that the appropriate sample preservation methods were used for tissues collected for intended testing (i.e. Histological samples fixed in Davidson's or other recommended fixatives, transport media, handling methods, etc.)
    - d. Reviewing proper and timely shipment or transport, including proper temperature of samples to laboratory centers for processing.
  3. Validation of Field Analysis: this review will evaluate that field data obtained meets QA/QC criteria including reviews documenting instrument calibration (such as pH meters, thermometers – Appendix D), or use of commercial laboratory field test kits with the manufacturer's recommended QA/QC for on-site data collection.
- B. Laboratory Activities Review: the review of laboratory activities shall be conducted by the QA Coordinator 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 the USFWS Procedures Manual guidelines. 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 in the USFWS Procedures Manual 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 equipment left unaddressed by Standard Operating Procedures (S.O.P.) Other objectives of this review are to identify proper use and maintenance of laboratory apparatus. This shall include but not be limited to:
    - a. Assessing that the appropriate apparatus is used for the targeted analytical assay and instrument placement is suitably located.
    - b. Assessing equipment is adequate and meets the needs for the capacity of

- average number of samples analyzed.
- c. Assessing that analytical equipment and other apparatus used is periodically cleaned, inspected, and calibrated as deemed necessary.
4. Evaluation of Reagent and Standards Preparation: this review addresses that all reagents and standards are those that meet the specifications of the S.O.P. approved by the guidelines in the USFWS Handbook. The review also addresses that preparation and dilutions of these reagents/standards are properly recorded, appropriately labeled and stored in suitable containers at the recommended storage conditions, and that new batches are tested where indicated.
  5. Evaluation of Sample Preparation: this review will identify that samples requiring specific preparations (ELISA preps, PCR extractions) have been completed as recommended by the USFWS Handbook.
  6. Evaluation of Analytical Testing: this review addresses that laboratory personnel are following the appropriate S.O.P. approved by guidelines outlined in the USFWS Handbook for each analytical assay. Any assays performed by SOPs that are not addressed by the USFWS guidelines must reference the source of the methods used and verify the testing procedure as recognized and published methodologies. (An example where this would be justified would include use of a diagnostic assay preferred by a cooperative partner such as those suggested by the Whirling Disease Foundation for research.)
  7. Evaluation of Analytical Assay Data with respect to method detection limits (MDL), precision/accuracy establishments and calculating uncertainty in measurements:
    - 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.
- C. 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. QA/QC Coordinator). The results of this audit provide documentation of bias of the analytical process for that individual assay for the target pathogen.
1. Any results which prove unsatisfactory indicate poor assay performance, and corrective action is required.
  2. 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
- f. sample integrity (storage, processing, etc.)

#### IV. External Audit Plan

The external audit team shall consist of two USFWS fish health biologist members and two members external to the USFWS with knowledge of the activities to be reviewed. The term membership for each auditor shall consist of three years. This team shall conduct ~~semi-annual~~ reviews of three USFWS FHC per year. Each laboratory will receive an external audit approximately once every three years.

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. External reviews conducted by outside official or commercial partners will be considered a valuable resource tool for continued laboratory evaluation and improvement.

#### V. Proficiency and Ring Testing Program

- A. This program is designed to provide evaluation of FHC proficiency and/or relative accuracy in performing standard protocol of a particular assay through analyses of sets of blind samples (infection status of tissues known only to the supplier) submitted to all FHC's by an outside source.
- B. Samples will be process according to the appropriate protocols by each laboratory within a given time frame, and results reported to a designated individual (National QIC) for analysis.
  - 1. Results will be analyzed and compiled into a confidential report to each FHC, indicating level of proficiency, accuracy and revealing any outlying results, with any suggested corrective actions required.
    - a. Any results which prove unsatisfactory indicate poor assay performance, and corrective action is required.
    - b. Evaluation of assay performance problems should involve, at minimum, the determination of the following:
      - 1. reagent quality (storage, preparation, shelf-life)
      - 2. quality, source and performance of standards and controls
      - 3. equipment calibration and maintenance
      - 4. equipment performance
      - 5. personnel training
      - 6. sample integrity (storage, processing, etc.)
  - 2. Results from all FHC's will be compiled into one report (FHC identities will not be provided in this report) to demonstrate consistency between FHC assay performance and results, as well as identify assays which are not producing consistent results. This report will be compiled by the NQIC and forwarded to all FHCs for review.

## **VI. Conclusion**

Internal and External QA/QC audits and participation in proficiency testing programs (either internally or externally) is the key to establishing proficient performance of laboratories and diagnostic assays. All USFWS FHC strive to maintain proficiency in each diagnostic assay conducted, and demonstration of this ability through implementation of this QA/QC Program will further assay validation, national consistency and reputability of the USFWS fish health program.

**APPENDIX B**  
**List of Reference Laboratories**

|   |   |
|---|---|
| <p><b>Pisces Molecular, LLC</b> 5311 Western Avenue, Suite E<br/>Boulder, CO 80301<br/>John Wood, PhD jwood@pisces-molecular.com<br/>Phone 303-546-9300 Fax 303-546-9400<br/>Molecular Biology Diagnostic Techniques and<br/>Reference</p>  | <p><b>Washington Animal Disease Diagnostic Laboratory</b><br/>College of Veterinary Medicine Washington State<br/>University PO Box 647034 Pullman, WA 99164-7034<br/>Phone 509-335-9696 FAX 509-335-7424</p>   |
| <p><b>Micro Technologies, Inc.</b> 41 Main St. Richmond, ME<br/>04357<br/>Deborah Bouchard Phone 207-737-2637<br/>FAX 207-737-4504<br/>ISAv reference (USDA- certified)</p>   | <p><b>U.S. Fish and Wildlife</b> Warm Springs Fish Health<br/>Center 5151 Spring Street Warm Springs, GA 31830<br/>Norm Heil, Director<br/>Phone 706-655-3382 FAX 706-655-9034<br/>USFWS: Largemouth Bass virus reference</p>   |
| <p><b>U.S. Fish and Wildlife USFWS</b> California-Nevada Fish<br/>Health Center 24411 Coleman Hatchery Road<br/>Anderson, CA 96007<br/>Scott Foott, Director<br/>Phone 916-365-4271 FAX 916-365-7150<br/>USFWSs: Negative Reference Tissues for<br/><i>R.salmoninarum</i> ELISA</p> | <p><b>Department of Medicine</b> School of Veterinary Science<br/>University of California Davis, CA 95616<br/>Dr. Ron Hedrick<br/>Phone 916-752-3411</p>   |
| <p><b>U.S. Geological Survey - Biological Resources Div.</b><br/>National Fish Health Research Laboratory 1700<br/>Leetown Rd. Kearneysville, WV 25430<br/>Frank Panek, Director<br/>Phone 304-724-4430 FAX 304-724-4435</p>  | <p><b>University of Maryland</b> VA-MD Regional College of<br/>Veterinary Medicine Aquatic Animal Health Center 8075<br/>Greenmead Drive College Park, MD 20742-3711<br/>Ana M. Baya<br/>Phone 301-314 -6837 FAX 301-314- 6855</p>  |
| <p><b>U.S. Geological Survey - Biological Resources Div.</b><br/>Western Fisheries Research Center 6505 N.E. 65<sup>th</sup> St.<br/>Seattle, WA 98115<br/>Dr. James Winton<br/>Phone 206-526-6282</p>  | <p><b>Aquatic Animal Health Program</b> Dept. Microbiology<br/>and Immunology College of Veterinary Medicine Cornell<br/>University Ithaca, NY 14853<br/>Dr. Paul R. Bowser<br/>Phone 607-253-3365 FAX 607-253-3384</p>   |
| <p><b>Dept. of Fisheries and Allied Aquacultures</b> Auburn<br/>University Auburn, AL 36849<br/>John Grizzle, Ph.D.<br/>Phone 334 844 3474 FAX 334 844 9208</p>   | <p><b>Dept. Biomedical Sciences and Pathobiology</b> VA-<br/>MD Regional College of Veterinary Medicine Virginia<br/>Polytechnic Institute and State University Phase III,<br/>Duck Pond Dr. Blacksburg, VA 24061<br/>Stephen A. Smith, D.V.M., Ph.D.<br/>Phone 540-231-5131 FAX 540-231-6033</p> |
| <p><b>USDA Agriculture Research USFWS</b> Stuttgart<br/>National Aquaculture Research Center P.O. Box 860<br/>Stuttgart, AR 72160<br/>Andrew J. Mitchell, Ph.D.<br/>Phone 870-673-4483 FAX 870-672-7710</p>   | <p><b>American Type Culture Collection</b><br/>ATCC<br/>P.O. Box 1549<br/>Manassas, VA 20110-2209<br/>Phone (703)365-2718<br/>Fax (703)365-2730<br/>Caroline O'Farrell, Ph.D.<br/><a href="http://www.atcc.org">http://www.atcc.org</a></p>   |
| <p>OIE Reference Labs are available at the following<br/>website:<br/><a href="http://www.oie.int/fdc/eng/Diseases/en_reflablist.htm">http://www.oie.int/fdc/eng/Diseases/en_reflablist.htm</a></p>   | <p>USDA, APHIS contact information can be obtained from<br/>the following website:<br/><a href="http://www.aphis.usda.gov/vs/aqua/aquaphis.html">http://www.aphis.usda.gov/vs/aqua/aquaphis.html</a></p>  |

## **APPENDIX C**

### **Suggested Documentation and Forms**

#### **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
- ✓ General communications relating to Case Histories

#### **Documents**

- ✓ U.S. Fish and Wildlife Service Policy Manual
- ✓ U.S. Fish and Wildlife Service, Fish Health Handbook (all sections)
- ✓ Any other protocols employed
- ✓ Regional Fish Health Policies
- ✓ State 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 FHC Director)

#### **Computer files to Maintain**

- ✓ Case History Database
- ✓ Reagent and media inventory
- ✓ Supply inventory

**APPENDIX C (continued)**  
**QA/QC INTERNAL/EXTERNAL AUDIT FORMS**  
**Field and Sampling Checklist**

| <b>Ref.<br/>Sect. II</b> | <b>Quality Control Item or Procedure Noted</b>   | <b>Suitable<br/>v</b> | <b>Not Suitable<br/>(Explain)</b> |
|--------------------------|--|-----------------------|-----------------------------------|
| <b>D.1.</b>              | Samples are collected by, or under supervision of USFWS Fish Health Biologist or veterinarian.                 |                       |                                   |
| <b>H.2.b.</b>            | Sterile utensils, loops, containers, tubes, vials used where appropriate.                                      |                       |                                   |
| <b>H.2.a.</b>            | Samples labeled appropriately.   |                       |                                   |
| <b>H.2.c.</b>            | Proper use of disinfectants.   |                       |                                   |
| <b>H.2.c.</b>            | Proper means of disposal of contaminated waste.  |                       |                                   |
| <b>Protocol</b>          | Proper means of specimen collection  |                       |                                   |
| <b>H.2.a.</b>            | Sample vessels are appropriately labeled   |                       |                                   |
| <b>H.2.a.</b>            | Sample information recorded for case information.  |                       |                                   |
| <b>H.2.c.</b>            | Specimens, tissues, and media are kept cool where appropriate throughout necropsy.                             |                       |                                   |
| <b>H.2.b.</b>            | Tissue samples are obtained in a manner which avoids contamination.  |                       |                                   |
| <b>Protocol</b>          | Cross contamination between samples prevented  |                       |                                   |
| <b>Protocol</b>          | Cross contamination between lots prevented   |                       |                                   |
| <b>Protocol</b>          | Sample transfer from field to lab – samples packaged to avoid leakage and cross-contamination during transfer. |                       |                                   |

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

**Attach notes and documentation of audit.**

**APPENDIX C (continued)  
QA/QC INTERNAL/EXTERNAL AUDIT**

**Laboratory Documentation/Sample Tracking Checklist**

| <b>Ref. Sect. II</b> | <b>Documentation</b>   | <b>Suitable v</b> | <b>Not Suitable (Explain)</b> |
|----------------------|--|-------------------|-------------------------------|
| <b>B.2.</b>          | Approved Laboratory and Diagnostic protocols available to all staff.   |                   |                               |
| <b>F.1.</b>          | Case History information entered onto CHR appropriately.   |                   |                               |
| <b>F.1.</b>          | Case History Record form printout available with appropriate information and attachments for each case.            |                   |                               |
| <b>F.1.</b>          | All racks and/or vessels containing processed samples are labeled with CHR number – all tubes with sample/lot I.D. |                   |                               |
| <b>H.3.a.</b>        | Sample logs maintained in individual laboratories (bact., vir., etc)for each case.                                 |                   |                               |
| <b>F.2.b.</b>        | All data generated from all sample assays documented, in black ink, on sample data forms.                          |                   |                               |
| <b>F.2.c.</b>        | Results properly reported and dated on CHR, with appropriate sample data forms attached.                           |                   |                               |
| <b>C.2.</b>          | Technical Qualifications File on all personnel.  |                   |                               |
| <b>E.1.b.</b>        | Appropriate equipment labeled with date of last calibration/certification.   |                   |                               |
| <b>E.1.</b>          | Equipment maintenance log with minimum of 2 years record of certification/calibration:                             |                   |                               |
|                      | Laminar flow hoods   |                   |                               |
|                      | Fume hoods   |                   |                               |
|                      | Balances   |                   |                               |
|                      | Micro-pipettors  |                   |                               |
|                      | Spectrophotometers   |                   |                               |
|                      | Thermocyclers  |                   |                               |
|                      | Thermometers   |                   |                               |
| <b>F.3.</b>          | CHR maintained in FHC files for minimum of 7 years.  |                   |                               |
| <b>E.2.h.</b>        | Station Safety/Hazard Communication Plan   |                   |                               |
| <b>E.2.h.</b>        | Material Safety Data sheets readily available for all materials.   |                   |                               |
| <b>E.2.a.&amp;b.</b> | All reagent containers properly labeled.   |                   |                               |

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

**APPENDIX C (continued)**  
**QA/QC INTERNAL/EXTERNAL AUDIT**

**Laboratory and Assay Performance Checklist**  
**BACTERIOLOGY**

| <b>Ref. Sect. II</b>     | <b>Quality Control Item or Procedure</b>                                       | <b>Suitable v</b> | <b>Not Suitable (Explain)</b> |
|--------------------------|--|-------------------|-------------------------------|
| <b>H.3.e.</b>            | Media Preparation  |                   |                               |
| <b>H.3.</b>              | Gram staining procedures   |                   |                               |
| <b>Protocol</b>          | Biochemical procedures and systems (API/other)                                 |                   |                               |
| <b>H.3.e.5.</b>          | Use and maintenance of control isolates  |                   |                               |
| <b>Protocol</b>          | Antibiotic sensitivity procedures  |                   |                               |
| <b>Protocol</b>          | Confirmatory Procedures  |                   |                               |
| <b>Refer To Protocol</b> | Fluorescent Antibody Procedures:   |                   |                               |
|                          | Sample preparation, fixation and storage                                       |                   |                               |
|                          | Conjugate preparation and filter sterilization                                 |                   |                               |
|                          | Staining procedures  |                   |                               |
|                          | Positive/negative controls   |                   |                               |
| <b>H.3.b.</b>            | Proper disposal of wastes  |                   |                               |
| <b>H.3.b.&amp;c.</b>     | Overall aseptic technique, disinfection and sterilization procedures/equipment |                   |                               |

**VIROLOGY**

| <b>Ref. Sect. II</b>   | <b>Quality Control Item or Procedure</b>  | <b>Suitable v</b> | <b>Not Suitable (Explain)</b> |
|------------------------|---|-------------------|-------------------------------|
| <b>H.4.d.</b>          | Maintenance of stock cell lines-passage if confluent cell monolayers                |                   |                               |
| <b>H.4.d.5.</b>        | Semi-annual mycoplasma screening and virus susceptibility checks of fish cell lines |                   |                               |
| <b>H.4.b.6.&amp;7.</b> | Media preparation and contamination checks  |                   |                               |
| <b>Protocol</b>        | Optimum cell lines, incubation temperatures and times used                          |                   |                               |
| <b>Protocol</b>        | Centrifugation techniques   |                   |                               |
| <b>Protocol</b>        | Viral plate reinoculation procedures  |                   |                               |
| <b>H.4.a.4.</b>        | CPE examination frequency/virus identification                                      |                   |                               |
| <b>Protocol</b>        | Virus confirmation procedures   |                   |                               |
| <b>H.4.b.8.</b>        | Proper disposal of wastes   |                   |                               |
| <b>H.4.b.</b>          | Overall aseptic technique, disinfection and sterilization procedures/equipment      |                   |                               |

**APPENDIX C (continued)**  
**QA/QC INTERNAL/EXTERNAL AUDIT**

**Laboratory and Assay Performance Checklist**  
**PARASITOLOGY – Refer to Parasitology Protocols**

| <b>Quality Control Item or Procedure</b>                                       | <b>Suitable v</b> | <b>Not Suitable (Explain)</b> |
|--|-------------------|-------------------------------|
| 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 |                   |                               |

**ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)**

| <b>Ref. Sect. II</b> | <b>Quality Control Item or Procedure</b>                                       | <b>Suitable v</b> | <b>Not Suitable (Explain)</b> |
|----------------------|--|-------------------|-------------------------------|
| <b>H.5.c.</b>        | Reagent preparation and storage  |                   |                               |
| <b>H.5.f.</b>        | Quality of glassware (acid washed, dedicated) – no plastics                    |                   |                               |
| <b>H.5.e.</b>        | Sample preparation   |                   |                               |
| <b>H.5.g.</b>        | Utilization of positive/negative controls                                      |                   |                               |
| <b>H.5.g.</b>        | ELISA plate – use of appropriate standard controls and replicates              |                   |                               |
| <b>Protocol</b>      | Adherence to protocol  |                   |                               |
| <b>H.5.h.</b>        | Interpretation of results  |                   |                               |
| <b>Protocol</b>      | Confirmation   |                   |                               |
| <b>Protocol</b>      | Proper disposal of wastes  |                   |                               |
| <b>Protocol</b>      | Overall aseptic technique, disinfection and sterilization procedures/equipment |                   |                               |

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

**APPENDIX C (continued)  
QA/QC INTERNAL/EXTERNAL AUDIT**

**Laboratory and Assay Performance Checklist  
POLYMERASE CHAIN REACTION (PCR)**

| Ref.<br>Sect. II       | Quality Control Item or Procedure  | Suitable<br>v | Not Suitable<br>(Explain) |
|------------------------|--|---------------|---------------------------|
| <b>H.6.d.2.</b>        | Reagent preparation and storage  |               |                           |
| <b>H.6.a.4.i.</b>      | Dedicated pipetters  |               |                           |
| <b>H.6.a.4.i.</b>      | Aerosol barrier and/or positive displacement pipette tips utilized.                          |               |                           |
| <b>H.6.a.5.</b>        | Latex or nitrile gloves utilized and changed appropriately                                   |               |                           |
| <b>H.6.a.4.</b>        | Appropriate laboratory set-up  |               |                           |
| <b>H.6.a.4.</b>        | Clean bench-top before and after assay with appropriate solution, bench liners used          |               |                           |
| <b>H.6.b.</b>          | Sample handling/preparation  |               |                           |
| <b>H.6.c.</b>          | DNA/RNA Extraction procedures  |               |                           |
| <b>H.6.c.3.</b>        | Proper controls  |               |                           |
| <b>H.6.c.4.</b>        | Quantification of template   |               |                           |
| <b>H.6.d.2.</b>        | Master mix preparation: documentation of reagent lots and concentration                      |               |                           |
| <b>H.6.d.5.</b>        | Sample loading and controls  |               |                           |
| <b>Protocol</b>        | Proper handling of tubes between first and second rounds in nested PCR                       |               |                           |
| <b>H.6.d.6.&amp;8.</b> | Proper centrifugation  |               |                           |
| <b>Protocol</b>        | Thermocycler properly programmed, record of temperature verification and maintenance on file |               |                           |
| <b>H.6.d.9.</b>        | Gel lane documentation and loading   |               |                           |
| <b>H.6.d.10.</b>       | Gel photodocumentation   |               |                           |
| <b>Protocol</b>        | Interpretation of results  |               |                           |
| <b>H.6.d.11.</b>       | Proper storage and documentation of samples, templates and PCR products.                     |               |                           |
| <b>H.6.a.6.</b>        | Proper disposal of wastes  |               |                           |
| <b>H.6.a.</b>          | Overall aseptic technique, disinfection and sterilization procedures/equipment               |               |                           |

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

**APPENDIX C (continued)  
Forms**

**Record of Media Preparation – Virology**

| <b>Date of Preparation:</b>         |  |  |  | <b>Media Prepared:</b> |  |  |  | <b>Lot Number:</b> |  |  |  | <b>Sterility Check:</b> |  |  |  |
|-------------------------------------|--|--|--|------------------------|--|--|--|--------------------|--|--|--|-------------------------|--|--|--|
| <b>Prepared by:</b>                 |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| Component                           |  |  |  | Date Prepared          |  |  |  | Sterility Check    |  |  |  | Initial                 |  |  |  |
| ___ X EMEM                          |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| FBS                                 |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| NaHCO <sub>3</sub>                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| Buffer                              |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| Antibiotics Added:                  |  |  |  | -----                  |  |  |  | -----              |  |  |  | -----                   |  |  |  |
| 1.                                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| 2.                                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| 3.                                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| L-glutamine                         |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| <b>Total Amount Prepared:</b> _____ |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |

**Record of Media Preparation – Virology**

| <b>Date of Preparation:</b>         |  |  |  | <b>Media Prepared:</b> |  |  |  | <b>Lot Number:</b> |  |  |  | <b>Sterility Check:</b> |  |  |  |
|-------------------------------------|--|--|--|------------------------|--|--|--|--------------------|--|--|--|-------------------------|--|--|--|
| <b>Prepared by:</b>                 |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| Component                           |  |  |  | Date Prepared          |  |  |  | Sterility Check    |  |  |  | Initial                 |  |  |  |
| ___ X EMEM                          |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| FBS                                 |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| NaHCO <sub>3</sub>                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| Buffer                              |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| Antibiotics Added:                  |  |  |  | -----                  |  |  |  | -----              |  |  |  | -----                   |  |  |  |
| 1.                                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| 2.                                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| 3.                                  |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| L-glutamine                         |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |
| <b>Total Amount Prepared:</b> _____ |  |  |  |                        |  |  |  |                    |  |  |  |                         |  |  |  |

**APPENDIX D  
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.**

| <b>EQUIPMENT</b>                        | <b>EXTERNAL CALIBRATION INTERVAL</b> | <b>INTERNAL CHECK INTERVAL</b> | <b>PARAMETERS TO CHECK</b>                                    | <b>INSTRUMENTS REQUIRED</b>                                       |
|---|--------------------------------------|--------------------------------|---|---|
| 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 D EQUIPMENT LOGS

A separate file shall be maintained for each item of equipment, to contain updated information. The following is a template set of required records to be kept for all equipment used for testing samples and calibration:

### EQUIPMENT INFORMATION

**ITEM:**

**INVENTORY NUMBER:**

**SERIAL NUMBER:**

**MANUFACTURER'S NAME AND CONTACT:**

**SOFTWARE USED (NAME)?**

**CURRENT LOCATION OF EQUIPMENT:**

**CURRENT STATUS:**    In Use

In Repair     Out of Service (see attached documentation for details)

**Additional Items on File:**

- Manufacturers Instructions
- Maintenance Plan
- Equipment Log (maintenance and calibration)
- Calibration Reports, Certificates, Adjustments made
- Documentation of Damage, Malfunction, modification or Repair
- Other: \_\_\_\_\_

---

Signature

Date







## APPENDIX E 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 7<sup>th</sup> 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 (Chlorine) – Broad spectrum disinfectant for use on waste liquids, instrument disinfection, surface decontamination and emergency spill decontamination. Use as follows:
  - 200 ppm active sodium hypochlorite solution –exposure for 1 hour minimum
  - 5000 ppm active sodium hypochlorite solution – exposure for 10 minutes**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:
  - Chlorine: 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; Hnath, 1983 and on the web – <http://www.mcgill.ca/eso/newweb/lab/biosafe.htm> )

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