

CHAPTER 15

Quality Control and Internal Review Process

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I. Introduction

Service Regional Fish Health Centers (FHC), with direction from the Washington Office (WO) and in cooperation with stakeholders (federal agencies, states, Tribes, and aquaculture industry) initiated a National Wild Fish Health Survey in May 1997 to determine distribution of certain pathogens in fish in the wild. Survey objectives are: (1) to incorporate standardized diagnostic and data management methods, (2) identify target pathogens, fish species and habitats and (3) develop a systematic watershed approach. The purpose of this review process is to provide for internal self-evaluation, ensure quality assurance/quality control of Survey results, and as necessary or appropriate, revise or update the Survey on a regular basis in light of the results of monitoring activities.

II. Scope of Evaluation

A. Biological

- ✓ Bacteriology/Fluorescent Antibody Techniques
- ✓ Virology
- ✓ Parasitology
- ✓ Enzyme-Linked Immunosorbent Assay
- ✓ Polymerase Chain Reaction (PCR)
- ✓ Histopathology

B. Programmatic

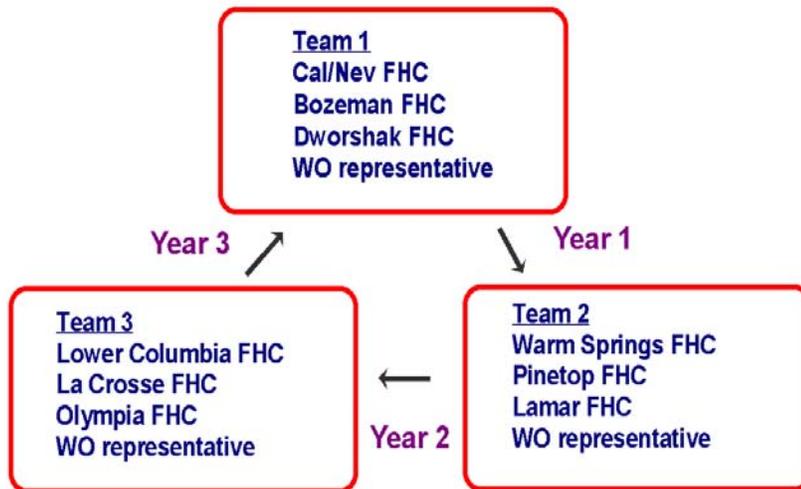
- ✓ Management staffing and training
- ✓ Data Verification/Information Transfer/Database Management
- ✓ Budget Verification

C. Operations

- ✓ Sample collection techniques
- ✓ Sample receipt and tracking
- ✓ Equipment and facilities
- ✓ Safety Program

EVALUATION TEAM

The nine national fish health centers participating in the Survey will be divided into evaluation teams as outlined below. The evaluation team will consist of one Washington Office representative or designate (the evaluation team leader) and one representative from each of the three fish health centers (FHC) in the team. Evaluations will be conducted throughout the fiscal year on a three-year rotational schedule as shown. In the first year, Team 1 will evaluate FHCs in Team 2, and in the second year, Team 2 will evaluate FHCs in Team 3, etc.



Final evaluation reports will be submitted by the team to the FHC project leader and Washington Office within 30 days of the on-site evaluation. Action items from the final report will be addressed by FHC within 60 days of evaluation. The evaluation process will be reviewed annually at the Fish Health Biologist meeting.

CHECKLIST FOR BIOLOGICAL EVALUATION

BACTERIOLOGY

Annual certification of laminar flow hood.

Suitable Not Suitable (state why) _____

Sample collection procedures.

Suitable Not Suitable (state why) _____

Media preparation (agar plates, tube media, etc.).

Suitable Not Suitable (state why) _____

Gram staining procedures.

Suitable Not Suitable (state why) _____

Biochemical procedures and identification system (Minitek, API systems, etc.).

Suitable Not Suitable (state why) _____

Expiration dates have not been exceeded on biochemical media and reagents used in bacteriology.

Suitable Not Suitable (state why) _____

Use of controls.

Suitable Not Suitable (state why) _____

Corroborative procedures.

Suitable Not Suitable (state why) _____

Positive controls.

Suitable Not Suitable (state why) _____

FLUORESCENT ANTIBODY TECHNIQUE

Sample collection procedures.

Suitable Not Suitable (state why) _____

Filtration of conjugate with 2Fm syringe filter.

Suitable Not Suitable (state why) _____

Positive control.

Suitable Not Suitable (state why) _____

Proper dilution of working conjugate (1:40 or as specified).

Suitable Not Suitable (state why) _____

Staining procedures and slide preparation.

Suitable Not Suitable (state why) _____

Slides examined with ultraviolet light with excitation and emission bands set as appropriate for FITC fluorescence (examine 50 fields per sample at 1,000x magnification).

Suitable Not Suitable (state why) _____

VIROLOGY

Sample collection procedures for fish tissue and ovarian fluid.

Suitable Not Suitable (state why) _____

Proper storage of fish tissue and ovarian fluid samples for viral assays.

Suitable Not Suitable (state why) _____

Maintenance of stock cell lines-passage if confluent cell monolayers.

Suitable Not Suitable (state why) _____

Long-term storage of tissue culture cell lines (i.e., in liquid nitrogen).

Suitable Not Suitable (state why) _____

Semi-annual mycoplasma screening of all fish cell lines.

Suitable Not Suitable (state why) _____

Semi-annual viral sensitivity checks on appropriate cell lines.

Suitable Not Suitable (state why) _____

Expiration dates have not been exceeded on tissue culture media and reagents used in virology.

Suitable Not Suitable (state why) _____

Preparation of media (HBSS and dilution blanks) and contamination detection.

Suitable Not Suitable (state why) _____

Washing and sterilization of glassware.

Suitable Not Suitable (state why) _____

Simultaneous seeding and preformed plate techniques.

Suitable Not Suitable (state why) _____

Optimum cell lines, incubation times and temperatures used for viral assays.

Suitable Not Suitable (state why)_____

Centrifugation of viral samples (temperature, time, rpms, etc.).

Suitable Not Suitable (state why)_____

Fetal bovine serum (FBS) growth check on cell lines and/or purchase of FBS from reputable supplier (e.g., Gibco, etc.).

Suitable Not Suitable (state why)_____

Viral plate reinoculation (reset) procedures for samples with toxicity.

Suitable Not Suitable (state why)_____

Isolate identification (frequency of examination and CPE of appropriate viruses).

Suitable Not Suitable (state why)_____

Procedures for viral confirmation.

Suitable Not Suitable (state why)_____

Proper storage and documentation of positive isolates (isolate archive).

Suitable Not Suitable (state why)_____

Laminar flow hood annual certification.

Suitable Not Suitable (state why)_____

PARASITOLOGY

Whirling Disease:

Sample collection/handling/storage.

Suitable Not Suitable (state why)_____

Pepsin-trypsin digest method protocols.

Suitable Not Suitable (state why)_____

Positive control spores.

Suitable Not Suitable (state why)_____

Suitable disinfection/cleaning between samples.

Suitable Not Suitable (state why)_____

Corroboration procedures suitable (PCR or histopathology).

Suitable Not Suitable (state why)_____

Reporting.

Suitable Not Suitable (state why)_____

Proper sample archive (10% formalin or histology slides) or photo documentation.

Suitable Not Suitable (state why)_____

Ceratomyxosis:

Sample collection/handling/storage (i.e., samples examined fresh).

Suitable Not Suitable (state why)_____

Protocols for presumptive diagnosis.

Suitable Not Suitable (state why)_____

Corroborative diagnosis of Cs.

Suitable Not Suitable (state why)_____

Reporting.

Suitable Not Suitable (state why)_____

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Bacterial Kidney Disease

Reagent preparation and storage (use of acid washed and/or disposable glassware, optimum dilutions determined by checker-board titration, sufficient replacement of buffer wash solution, etc.).

Suitable Not Suitable (state why)_____

Sample collection (disinfection between fish) and appropriate dilution in PBS-T20.

Suitable Not Suitable (state why)_____

ELISA preparation (i.e., micropipettes disinfected with ethanol, use separate micropipettes for positive control and sample tissues) and controls used (blank, conjugate, substrate-chromogen, and negative and positive kidney antigens).

Suitable Not Suitable (state why)_____

Proper use of standards: positive and negative control tissue and diluent (PBS-T20) for blank wells.

Suitable Not Suitable (state why)_____

Interpretation of ELISA results.

Suitable Not Suitable (state why)_____

Documentation of assay steps and adherence to protocol.

Suitable Not Suitable (state why)_____

Corroborative testing (PCR) procedures followed (three samples with highest OD values per case tested by Rs-PCR).

Suitable Not Suitable (state why)_____

Operation/maintenance of equipment.

Suitable Not Suitable (state why)_____

Reporting.

Suitable Not Suitable (state why)_____

POLYMERASE CHAIN REACTION (PCR)

Work areas separated for DNA extractions, PCR set-up and dispensing or aliquoting PCR reagents. Containment units (i.e., biosafety cabinets, hoods) should be used.

Suitable Not Suitable (state why)_____

Separate supplies and pipetting devices should be used in each work area. Positive-displacement or filtered pipette tips used.

Suitable Not Suitable (state why)_____

Laboratory precautions (typical of handling radioactive substances) and decontamination of work surfaces and supplies.

Suitable Not Suitable (state why)_____

Reagents prepared, aliquotted and stored in area free of PCR-amplified product. Reagents aliquoted to minimize repeated samplings. Lots of reagents used recorded.

Suitable Not Suitable (state why)_____

Sample collection/handling/storage.

Suitable Not Suitable (state why)_____

Sample processing procedures (i.e., gloves changed frequently, decontamination of instruments)

Suitable Not Suitable (state why)_____

Use of controls [small number of positive controls with low target molecule concentration, a large number of both reagent controls (i.e., reactions lacking only template DNA) and pathogen negative controls].

Suitable Not Suitable (state why)_____

Reporting (photo documentation of gels) and archiving of positive samples.

Suitable Not Suitable (state why)_____

CHECKLIST FOR PROGRAMMATIC EVALUATION

MANAGEMENT STAFFING AND TRAINING

Staffing is sufficient to carry out survey objectives.

Suitable Not Suitable (state why) _____

New employees should be trained on procedures and equipment use before performing assays independently. A training record is maintained and signed off by employee and supervisor.

Suitable Not Suitable (state why) _____

DATA VERIFICATION/INFORMATION TRANSFER

Data Verification

Lab and field data as generated and/or documented by the Fish Health Centers will be verified by an authorized person (the AVerifier) prior to entry into the national database.

Suitable Not Suitable (state why) _____

Verifier authorization will be given to the FHC Project Leader(s) for each center. Additionally, the Project Leader can also empower immediate support staff persons to verify information on their behalf.

Suitable Not Suitable (state why) _____

Authorized verifiers must be familiar with the requirements of the National Fish Database and be given a formal briefing about the significance of each of the following five elements of the database: 1. field sampling, 2. chain-of-custody, 3. diagnostic test, 4. data base field and 5. information transfer (formal training may be required).

Suitable Not Suitable (state why) _____

Authorized verifiers will examine each of the five elements and check-off the appropriate box for conformity or non-conformity. If the nonconformity box is checked, a note must be placed in the appropriate note field. (required).

Suitable Not Suitable (state why) _____

Authorized verifiers will check-off the appropriate box for each of the five elements, make notes where appropriate, and sign with their Verifier ID# (or name).

Suitable Not Suitable (state why) _____

Information Transfer

Field Samples identified confirmed to be positive for a certain pathogen (determined to be important for species/pathogen management) will be identified by FHC. Early notification of these results will be provided to the state/federal resource agencies having jurisdiction over the geographic areas that might be affected if a certain fish pathogen were detected in the sample watershed. Additionally, the WO Liaison for FHCs and the Regional Fisheries ARD will also be notified of the finding. No data regarding such a sample will be entered into the National data base for up to 15 days from the date of verification, or sooner if the specific resource management agency that might be affected by the finding approves data entry. All correspondence will be documented.

Suitable Not Suitable (state why) _____

BUDGET VERIFICATION

Suitable records of survey expenses should be maintained. Allowable cost categories for planning and budget include:

Capital equipment cost (should be minimal).

Suitable Not Suitable (state why)_____

Diagnostic Tests - in lab supplies and labor (75% of annual funding)

Suitable Not Suitable (state why)_____

Technical Assistance - Labor costs associated with providing advice and technical support to cooperators; WFS Conferences, meetings and stakeholder outreach

Suitable Not Suitable (state why)_____

Field sampling/collection costs - supplies and labor outside of the lab (<25% of annual funding spent on collection)

Suitable Not Suitable (state why)_____

Data Verification/Data Entry - Labor for data entry into database.

Suitable Not Suitable (state why)_____

Travel- airfare, common carrier and other expenses identified on a voucher (motel, per diem, etc.), not to include labor.

Suitable Not Suitable (state why)_____

CHECKLIST FOR OPERATIONS EVALUATION

SAMPLE RECEIPT AND TRACKING

Chain-of-Custody

An accurate case history report and sample collection record shall accompany all samples.

Suitable Not Suitable (state why) _____

All samples labeled with appropriate unique case history number.

Suitable Not Suitable (state why) _____

All data generated during sample processing shall be documented, in black ink, on the case history report. Pertinent entries shall be dated and initialized by the employee who performed the work. Any changes to the original entry shall not obscure the original entry. The reason for change should be indicated, dated, and initialed by the employee performing the change. Automated data records should adhere to similar standards.

Suitable Not Suitable (state why) _____

Any samples transferred to an outside source for examination, or final disposition will be documented on the case history report, and a signed chain-of-custody form will accompany the items to the new destination. A copy of the chain-of-custody form will be attached to the case history report.

Suitable Not Suitable (state why) _____

Records associated with case history reports shall be retained no less than 7 years.

Suitable Not Suitable (state why) _____

Each center shall follow Survey protocols and work with other centers to review and revise as necessary.

Suitable Not Suitable (state why) _____

Equipment logs shall be maintained no less than 2 years.

Suitable Not Suitable (state why) _____

EQUIPMENT AND FACILITIES

Center facilities should be separated into specific zones for sample processing (i.e., handling fish), administrative activities and laboratory testing.

Suitable Not Suitable (state why) _____

Laboratory equipment shall be adequately inspected, cleaned, and maintained in an efficient operating condition at all times, and if required, regularly serviced by qualified professionals.

Suitable Not Suitable (state why) _____

Equipment used for generating measurements shall be calibrated and/or standardized prior to use as specified in the manufacturer's handbook or as deemed sufficient by the evaluation team. A log containing dates these activities were performed, as well as any repairs or defects will be maintained.

Suitable Not Suitable (state why) _____

Center facilities shall use approved procedures for disposal of samples and laboratory wastes.

Suitable Not Suitable _____

Records associated with case history reports shall be retained no less than 7 years.

Suitable Not Suitable (state why) _____

SAFETY PROGRAM

Station safety plan.

Suitable Not Suitable (state why) _____

Hazcom plan.

Suitable Not Suitable (state why) _____

Chemical hygiene plan

Suitable Not Suitable (state why) _____

Hazard chemical inventory list.

Suitable Not Suitable (state why) _____

First aid kit in building plan

Suitable Not Suitable (state why) _____

Material safety data sheet station.

Suitable Not Suitable (state why) _____

Eye wash station in each lab.

Suitable Not Suitable (state why) _____

Annual fume hood certification.

Suitable Not Suitable (state why) _____

NFPA identification system displayed in building.

Suitable Not Suitable (state why) _____

Properly labeled chemical flammable storage unit.

Suitable Not Suitable (state why) _____

Building fire evacuation plan.

Suitable Not Suitable (state why)_____

Chemical spill clean-up kit.

Suitable Not Suitable (state why)_____

Hazardous chemical waste disposal system.

Suitable Not Suitable (state why)_____

Infectious waste-sharps container in each lab.

Suitable Not Suitable (state why)_____

Personal protective equipment (glasses, aprons, rubber gloves, ear plugs, etc.).

Suitable Not Suitable (state why)_____

Fire extinguishers in each lab.

Suitable Not Suitable (state why)_____

IV. References

National Wild Fish Health Survey, Protocols & Procedures. May 1997. Division of Fish Hatcheries, D.C.

Quality Control/Quality Assurance Program for U.S. Fish & Wildlife Service, Fish Health Centers. June 1992. Draft II.

Fisheries Resources, Technology Center Evaluation Program, October, 1994, Draft Version #4.