

1.1 Introduction

A. Purpose

The purpose of this document is to serve as a set of standardized testing protocols to be followed when carrying out health inspections on aquatic animals. Using standardized procedures and protocols allows fisheries biologists and managers to better compare data and make more informed management decisions when evaluating the intrastate, interstate, or international movement of aquatic animals. However, the final decision to test for specific aquatic pathogens remains in the hands of those regulating bodies requesting/requiring aquatic animal health inspections. When a regulating body requests/requires testing for specific aquatic pathogens the standardized protocols included in Section 2 of the AFS Blue Book should be utilized to provide comparable test results. The aquatic pathogens listed in Section 2 of the AFS Blue Book are in no way intended to establish, dictate, or supplant the aquatic animal health policies of any regulating body. The pathogens listed and their corresponding protocols are included solely to provide standardized testing methods that produce comparable data. Inclusion of pathogens in Section 2 of the AFS Blue Book is not meant to suggest that testing for all listed pathogens is appropriate or required in all situations or in all aquatic species (see section D. “Selection of Pathogens” below).

B. Composition

This handbook reflects the combined efforts and expertise of the United States Fish and Wildlife Service Fish Health Centers and the American Fisheries Society Fish Health Section. It has been assembled by a vast array of individuals with academic and field expertise. It is a compilation of methodologies determined to be most appropriate for detecting the presence of specific pathogens during an aquatic animal health inspection. The methodologies are based on numerous sources, including the United States Fish and Wildlife Service’s National Wild Fish Health Survey Manual, the American Fisheries Society Blue Book, the 5th edition of the Office International des Epizooties (OIE) Manual of Diagnostic Tests for Aquatic Animals, Alaska Department of Fish and Game, Fish Pathology Section Laboratory Manual, and the peer reviewed literature. Without the substantial contributions of these documents and the individuals that assembled and bench tested them, the development of this handbook may not have been possible.

C. Design

To be effective and to meet the needs of what is a rapidly changing and expanding field, this handbook must be extremely dynamic. Every section, including this first chapter, is open to revision. The design of this handbook will allow it to grow and change as time progresses and standardized inspection techniques are required for additional pathogens or better testing methods are made available. The guidelines for introducing changes are outlined in Section 2, A1 Appendix 1. Section 2, A3 Appendix 3 summarizes the decision making process for the selected assays and pathogens so that future changes can be made efficiently.

D. Selection of Pathogens

The pathogens considered in this handbook are those that meet the following criteria:

- a. They have the potential to produce severe epizootics of clinical disease.
- b. They are known to exist subclinically in apparently healthy fish.
- c. There are state, federal, or international requirements that fish be inspected for the pathogen.
- d. There are both screening and confirmatory tests available that are effective for the detection and identification of each pathogen in the absence of clinical signs.

Inclusion of pathogens in this document is not meant to imply that it is appropriate to test for all pathogens in all situations. Testing requirements should be based on a careful regional assessment of the risks posed by particular pathogens. The protocols in this document should then be used to test susceptible species for pathogens deemed to be of importance in that region.

E. Selection of Methodologies

The appropriateness of methodologies was determined based not only on performance characteristics (sensitivity, specificity, repeatability, and reproducibility) but also on the appropriateness in a given species or population, scientific acceptance and citable reference materials, cost, availability of reagents, availability of technology, time necessary to process samples and time required before a report can be issued, sample type and its viability, manpower requirements, number of samples to be done, the existence of reference standards, and safety.

The assays specified in this handbook are of two types. Generally, the screening method is one with a proven track record for isolation of a fish pathogen or, less commonly, for direct observation of a causative agent or detection of a component of a fish pathogen. In many cases (e.g. cell culture isolation of fish viruses), these were the first methods developed for this purpose. Although many of these assays have been used for sufficient periods of time to engender confidence in their utility, few if any of the screening methods have been subjected to the formal validation process outlined by the OIE (see “Validity of Chosen Methodologies”). Typically, this is because for many years, few competing assays were available for the purpose; however, there remains a need for the validation process to be applied to these screening tests. Nevertheless, specifying a single screening method ensures that the sensitivity of the assay used to detect a pathogen, while not known with precision, is relatively uniform wherever the assay is applied.

The second type of assay is a confirmatory, test used to verify the identity of a suspect agent. These assays are also in need of formal validation, but are used here only to accurately identify an agent isolated in culture or identified visually, with less regard as to their absolute level of sensitivity. Various assays may be appropriate as confirmatory tests if the specificity of each assay is high. Knowledge of the sensitivity of a confirmatory test becomes of increased importance when the assay is used to confirm a screening test that does not propagate the pathogen in culture. An example is the use of a DNA-based test (e.g. PCR) to confirm a serological test (e.g. FAT). In this case, both assays detect proxies for the actual agent. Better information on the sensitivity, specificity, repeatability and reproducibility of these assays would allow fish health workers to choose optimal combinations of such proxy assays.

F. Validity of Chosen Methodologies

Validation is the evaluation of a process to determine its fitness for a particular use. For diagnostic assays, this generally involves measuring the ability of a certain test to accurately predict the infection status of an animal from which a sample was obtained. Naturally, many factors affect the ability of any assay to achieve absolute predictive power. In recent years, the development of assays based upon molecular approaches and the increased attention paid to issues of quality assurance have led to concerns about the relative performance of various diagnostic assays for fish diseases.

The principles involved in validation of diagnostic assays for infectious diseases are included as a chapter in the Manual of Diagnostic Tests for Aquatic Animals published by the Office International des Epizooties (OIE) in Paris, France. This material is available on the web (www.oie.int) and in hard copy from the OIE. In addition to presenting the principles involved, the OIE lists five stages for diagnostic assay validation and discusses these in some detail with references for further reading. The chapter also describes the procedures by which the important parameters of sensitivity, specificity, repeatability, and reproducibility are determined within a known degree of statistical precision.

Finally, as new diagnostic tests for fish diseases become available, there is interest in comparing the relative performance of various assays. Formal validation methods provide a basis to accurately quantify the actual performance of assays used in fish health and provide information that can help to determine when newer assays should replace the established assays as screening or confirmatory methods. Validation methods can also resolve uncertainties as to the relative performance of materials from different commercial sources or molecular assays that are similar in type, but target different proteins or genes of a pathogen or that use different protocols or reagents. Unfortunately, few if any fish disease detection assays have ever been validated in this way. While validation testing involves much work, considerable expense, and the assembly of sets of standard samples of known infection status, the fish health community is very much in need of this information.

G. Other Important Considerations

It should be noted that as with any inspection manual or handbook, the techniques employed provide only a snapshot in time. There is no guarantee that any inspected animal or population of animals is disease free. The only statement that can be made is that at the time the animals were sampled the disease organism was not detected by the testing methods utilized. Additionally, this handbook cannot cover every scenario that may occur. For this reason, it remains the obligation and responsibility of the individual inspector to determine what the best sampling protocols are for a given inspection and that any inspection methods or protocols used meet the requirements of the requesting entity.

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