A3.5 Parasitology Position Statements

A. 2000 – 2002 Position Statements

1. Initial Position Statement

The pathogens selected were those the committee felt were of the greatest regulatory importance at the time the handbook was being developed. Rationale for selection of the screening and confirmatory assays for each of the fish parasites considered in Section 2, Chapter 5 Parasitology are detailed below. Confirmatory procedures will only be used if the sample is presumptively positive using the approved screening method. Please refer to Section 1, Chapter 1 Introduction for explanation of the acceptance of non-validated procedures for confirmation.

a. ***Myxobolus cerebralis***

i. Screening

The pepsin-trypsin digest procedure was selected as the assay of choice for isolation and concentration of spore stages from fish cartilage. Although it was acknowledged that the plankton centrifuge method offers some advantages in the ease of assay performance, review of the literature and of laboratories performing *M. cerebralis* diagnostics supported selection of the digest assay for reasons of increased sensitivity. The procedure does allow pooling of up to five fish, which is likely to decrease detection sensitivity. However, it was considered that processing of individual fish would constitute a workload beyond the capability of many laboratories, and that in some regions of the country this would be considered unacceptable. The decision was to allow pooling with the realization that in areas most affected by the parasite, there would be requirement by the states to process single fish.

ii. Confirmation

Confirmation is either by identification of spores in histological sections or detection of parasite DNA by polymerase chain reaction (PCR) assay. Detection in histological sections is the current standard. Although the committee felt that it is of lower sensitivity than the PCR assay, it will remain an acceptable confirmatory tool at this time. For DNA detection, the nested PCR assay was selected because it is scientifically acceptable and citable and it is used successfully in a number of laboratories. Because the sampling and preparation procedures described in the original publication were primarily for research purposes, the protocol described here references methods more in line with those required during field collections of fishes of different sizes. These collection and preparation methods are compatible with performing the nested assay.

b. ***Ceratomyxa shasta***

i. Screening

Presumptive identification is based on identification of any parasite stages in wet mount scrapings, the procedure currently recommended.

ii. Confirmation
Because of the distinctive morphology of the *C. shasta* spore, its identification is sufficient for confirmation. If spores are not identified, a presumptive positive can be confirmed by detection of the parasite DNA by PCR. The protocol described is published and has been developed for diagnosis in field situations. Other confirmatory procedures requiring monoclonal antibodies were not considered because these reagents are not commercially available.

c. ***Tetracapsula bryosalmonae***

i. Screening
Presumptive identification is made by identifying any parasite in stained imprints or using lectins. These two methods were proposed because identification of the parasite is difficult without practice, and the lectin has been shown to increase detection.

ii. Confirmation
At this time, confirmation is by identification of any parasite stages in histological sections. Although this method is not highly sensitive and requires a trained eye, it was agreed that scientific review of other methods made them unfeasible at this time. The lectin stain has been demonstrated to cross-react with other myxozoans and there is also question about the specificity of published PCR assays. The committee felt that this protocol would probably be updated in the near future as a demonstrated specific PCR assay becomes available.

d. ***Bothriocephalus acheilognathi***

i. Screening
Presumptive identification is by identification of basic characteristics of the cestode.

ii. Confirmation
Presumptive cestodes are confirmed by identification of key morphological characteristics. These visual identification methods are accepted in the scientific literature and are the current Blue Book standard.

B. 2002 – 2003 Position Statements

1. Review use of digest material for PCR confirmation of *Myxobolus cerebralis*.

a. Adoption of the nested PCR technique on digest material for confirmation of the presence of *Myxobolus cerebralis* can be scientifically defended at this time. Baldwin and Myklebust's work statistically determined sensitivity of single round PCR from pooled digest material from infected and non-infected reference animals, and added additional information regarding specificity. Though statistically significant, the number of samples examined was quite low, and although not determined, the confidence limits for sensitivity and specificity would likely be quite large. Qureshi et al. examined a large number of clinical samples (580 fish) using nested PCR on the digest product and compared results with the current gold standard, histologic examination, as well as with the tissue digest. Testing of additional animals should be done and levels of sensitivity need to be determined for the nested procedure applied to

digest material, but there is already more information available on this assay than for almost any other test.

2. **Review histological confirmation of *Myxobolus cerebralis.***

   a. The committee recommended that the criteria for determining a sample negative by histology be made more stringent. Wording will be changed to include serial step sections in samples where the initial sections examined were negative, and inability to detect any spores in tissue will no longer be considered sufficient to certify a lot of fish as negative.

3. **Review protocols allowing freezing of samples for *Myxobolus cerebralis* spore recovery.**

   a. The committee recommended that no change be made to the current procedure, which allows PTD processing of frozen samples with modifications of enzyme concentrations. There is insufficient peer-reviewed scientific data to prove and quantify the effects of freezing on spore recovery and requiring processing of fresh heads would present a problem for many laboratories.

4. **Review of PCR diagnosis of *Tetracapsula bryosalmonae*.**

   a. The committee agreed that recent publications on this assay demonstrate that it is a valid confirmation test and this will be added as an alternative to histology. Concerns about this and other PCR assays continue to be QA/QC issues like availability of positive control tissues.

C. 2003 – 2004 Position Statements

The full committee voted to include a modification of the whirling disease enzymatic digestion mixture to include a pH indicator. This mixture has been widely used for many years and will function at least as well as the current mixture.

D. 2004-2005 Position Statements

a. Is it possible to sample for *Tetracapsula bryosalmonae* and *Ceratomyxa shasta* in a manner similar to methods used for *M. cerebralis*?

b. Rather than sampling every lot at a hatchery for these pathogens, could only the most susceptible lot be sampled; and could samples be pooled (a 5-fish pool was suggested)?

c. No comparisons have been made regarding the detection of *T. bryosalmonae* or *C. shasta* using the most susceptible lot vs. sampling all the lots at a hatchery.

d. Nor have experiments been done to show that processing samples for screening *T. bryosalmonae* and *C. shasta* as 5- fish pools will demonstrate the true health status (positive or negative) of a lot of fish compared to sampling individual fish.

e. Based on the absence of data, the Oversight Committee agreed no changes to sampling methods for *T. bryosalmonae* and *C. shasta* will be made at this time.

E. 2006 – 2007 Position Statements

No changes or reviews requested.

E. 2008 – 2010 Position Statements

1. Should a *Myxobolus cerebralis* PCR protocol be used in the AFS-USFWS Standard Procedures for Aquatic Animal Health Inspections as a screening tool?

While the committee recognized that there may be a need for a more sensitive assay with fewer time restrictions, we decided that the inclusion of PCR as a screening tool was unacceptable at this time for the following reasons: 1. The primer set with a peer reviewed, published validation study is under patent, and the company that owns the patent will not allow use of the primers outside their lab 2. The alternative primer set (the HSP-70) proposed has no peer reviewed validation publication.