Laboratory validation of real-time polymerase chain reaction (qPCR) analysis for *Myxobolus cerebralis*

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ABSTRACT:

Accurate detection of *Myxobolus cerebralis*, the causative agent of Whirling Disease in salmonids, was compared using three previously developed real-time PCR methods (qPCR). A combination of intra and inter-laboratory analysis using synthetic gBlocks along with naturally and experimentally infected fish tissue was used to evaluate assay performance. Additionally, isolates that represent geographically distinct locations were tested by all three assays. Findings demonstrated that the Kelley 18S (2004) and Cavender 18S (2004) protocols provided high assay sensitivity, repeatability of replicates within and between reactions and reproducible identification of all test samples across multiple laboratories. The Kelley 18S protocol was the only assay that also demonstrated 100% detection accuracy when testing geographically distinct *M. cerebralis* isolates. We determined that the Kelley 18S protocol was robust under variable test conditions (3 thermocylers, 5 laboratories, multiple operators, and varied sample type), was more sensitive than the Cavender 18S and Hsp70 methods, and provides equivalent detection capabilities to the currently accepted nPCR molecular confirmation method "gold standard" for *M. cerebralis* described in the American Fisheries Society-Fish Health Section Blue Book. Results from this work demonstrate the Kelley 18S qPCR protocol provides a good "fit for purpose" approach for *M. cerebralis* surveillance and confirmation methods.