

Comparison of Nested and Single Round Polymerase Chain Reaction Assays for Detection of *Myxobolus cerebralis* in Rainbow Trout

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STUDY OBJECTIVES:

1. Determine the relative effectiveness of the Single Round PCR assay to detect *Myxobolus cerebralis* rainbow trout exposed to controlled doseages of TAMs as compared to the nested PCR assay.
2. Establish Method Detection Limits for both the Single and Nested PCR assay.
3. Compare the relative effectiveness of a tissue extraction or parasite spore extraction for detection of *Myxobolus cerebralis*.
4. Individual Fish vs. 5 fish pooled samples?

STUDY DESIGN:

1. 5 week old post-hatch Arlee strain rainbow trout will be utilized for TAM exposures.
2. 180 (5 week old post-hatch) rainbow trout fry will be exposed to 1,000 TAMs/fish.
3. 60 fish will be used as a sham-exposure control.
4. Fish will be equally divided into 10 gallon aquaria and held at the Wild Trout Research for approximately 150 days for the development of mature parasite spores in cartilage.
5. 2 replicates of 60 fish each will be sampled as individual head samples. =120 fish total
6. 2 replicates of 60 fish will also be combined into 5 fish pool (12 samples).=24 samples total
7. All fish heads will be cut bisected sagittally. Assay will be performed on random half head sample.
8. The Pepsin/Trypsin digest protocol per AFS/Blue Book will be performed on half heads.
9. Spore digest will be used to count spores. Number of spores per half head will be recorded. Dilutions of spore digest will be made to determine method detection limits with known spore loads per fish.
10. The presence of mature *Myxobolus cerebralis* parasite spores in cranial cartilage will be confirmed with histology and the infection graded as per MacConnell/Baldwin scale.
11. Both the single round and nested assays will be performed on the spore digest dilutions to establish a detection limit.

12. PCR assay runs will be limited to 20 fish per run.