

**Assay Validation of Quantitative Polymerase Chain Reaction (QPCR) for detection of *Renibacterium salmoninarum* using a Positive PCR Control with known quantity of the msa gene.**

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Quantitative Polymerase Chain Reaction (QPCR) is a powerful molecular tool being utilized by the U.S. Fish and Wildlife Service (USFWS) Fish Health Centers in Region 1. QPCR is both a highly specific and sensitive detection tool which utilizes specially integrated instruments, powerful software and Taqman® fluorescent probes to measure small quantities of pathogen DNA in fish tissues. This study focuses on the detection of *Renibacterium salmoninarum* DNA, specifically the correlation of Cycle Threshold (Ct) values obtained with QPCR to standard detection methods such as bacterial culture on KDM media, cell counts by membrane-filtration Fluorescent Antibody Test (mf-FAT) and optical density (OD) values obtained with the Enzyme Linked Immunosorbent Assay (ELISA). DNA extraction efficiency, QPCR assay sensitivity and correlation with other testing methods are evaluated using cultured *Renibacterium salmoninarum* (ATCC#33206) and known Rs-positive kidney tissue from chinook salmon (*Oncorhynchus tshawytscha*). This study utilized and evaluated a new *Renibacterium salmoninarum* positive control for PCR, a DNA plasmid, developed by Dr. Caroline O'Farrell of the American Type Culture Collection (ATCC). The plasmid, with known quantities of the Rs msa gene, permitted quantitative comparison of QPCR with the standard detection methods used to detect *Renibacterium salmoninarum*.