

3.1 Bacteriology Introduction

The following chapter describes inspection procedures for bacterial pathogens of fish that may be required for a fish health inspection. The target bacterial species include *Aeromonas salmonicida*, *Yersinia ruckeri*, *Edwardsiella ictaluri*, *Renibacterium salmoninarum*, and *Piscirickettsia salmonis*. [Section 2, Chapter 2 Sampling](#) describes procedures for proper sampling of fish tissues to ensure detection of any of these pathogens during a fish health inspection.

Presumptive identifications of *A. salmonicida* (subspecies *salmonicida* and *achromogenes*) ([Section 2, 3.2 *Aeromonas salmonicida* \(Furunculosis\)](#)), *E. ictaluri* ([Section 2, 3.4 *Edwardsiella ictaluri* \(Enteric Septicemia of Catfish, ESC\)](#)), and *Y. ruckeri* ([Section 2, 3.3 *Yersinia ruckeri* \(Enteric Redmouth Disease, ERM\)](#)) are based on Gram staining properties, and characteristic biochemical reactions. Confirmatory identification consists of fluorescent antibody testing using fluorescein-conjugated, species-specific antibody ([Section 2, 3.8.E “Fluorescent Antibody Test \(FAT\)”](#)). Known isolates of *A. salmonicida*, *E. ictaluri*, and *Y. ruckeri* are purchased from ATCC and are used as positive controls. Single, unknown isolates may be used to test for all three of these organisms.

The presumptive identification of the Gram-positive bacterium *R. salmoninarum* ([Section 2, 3.5 *Reibacterium salmoninarum* \(Bacterial Kidney Disease, BKD\)](#)) is based upon serological methods. For purposes of initial screening and detection of the pathogen, the direct fluorescent antibody technique (FAT) on kidney smears and ovarian fluid samples is employed ([Section 2, 3.8.E “Fluorescent Antibody Test \(FAT\)”](#)). Documentation exists which indicates the possibility for false positive results caused by bacterial organisms which cross react with antibodies prepared against *R. salmoninarum* (Austin et al., 1985; Bullock et al., 1980; Brown et al. 1995). For this reason, it is important to follow steps described below to confirm that a positive FAT result is due to the presence of this pathogen. **Exception:** Anadromous salmonids regularly monitored for *R. salmoninarum* with ELISA or quantitative PCR techniques may be considered positive without additional testing by FAT.

Any FAT results which appear positive for *R. salmoninarum* should be confirmed by either culture of kidney tissue on selective kidney disease medium (SKDM-2) ([Section 2, 3.5.B.1 “Bacterial Culture”](#)) or by testing the positive tissues with the polymerase chain reaction (PCR) technique ([Section 2, 3.5.B.2 “Nested Polymerase Chain Reaction \(PCR\) for Confirmation of *R. salmoninarum* DNA”](#)).

The presumptive identification of the gram-negative, intracellular bacterium *P. salmonis* ([Section 2, 3.6 *Piscirickettsia salmonis*](#)) is based on isolation in tissue cell culture without antibiotics and/or detection in stained tissue impressions ([Section 2, 3.6.A “Summary of Screening Tests”](#)). Confirmatory testing is by serological methods or PCR ([Section 2, 3.6.B “Confirmatory Tests”](#)).

DISCLAIMER: Mention of specific brands or manufacturers does not warrant endorsement by the U. S. Fish and Wildlife Service, the United States government, and/or the American Fisheries Society. Any comparable instrument, laboratory supply, or reagent may be substituted in this protocol if operation and performance are deemed comparable to the items specified.