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.