

3.2 *Aeromonas salmonicida* (Furunculosis)

Aeromonas salmonicida may be difficult to detect based on a variety of environmental, physiological, and host factors. At water temperatures between 14 to 24°C, fish can develop clinical disease within 4 to 12 days after bacterial exposure in the water supply. At temperatures below 13°C, chronic and latent infections are more likely to develop. This pathogen has been associated with disease in a variety of salmonid and non-salmonid species. It is generally accepted that any freshwater fish can carry the bacteria with or without showing signs of disease (Bullock et al. 1983; Thoesen 1994). The typical form of the bacterium (subspecies *salmonicida*) produces a soluble brown pigment on tryptic soy agar after two to three days of growth at 20°C. The less common and atypical strain of this pathogen (subspecies *achromogenes*) does not produce pigmentation under these incubation conditions. Both strains, however, have been associated with disease in a variety of fish species (Paterson et al. 1980).

A. Summary of Screening Test

1. Bacterial Culture and Biochemical Analysis

- a. Aseptically inoculate samples into TSA tubes or onto plates as described in Section 2, 2.2 Sampling.
- b. Incubate for 24 to 48 hours at 20 to 24°C (room temperature). If no growth occurs at either 24 or 48 hours, record this information on the data sheet. **If no growth occurs after 96 hours, samples are reported as negative for *A. salmonicida*.**
- c. When primary culture occurs on tubes or plates use a sterile loop or needle to select a single, isolated colony to subculture onto fresh TSA plates. If colonies are not well isolated on the original media, a new plate will have to be streaked over the entire plate surface to achieve isolation of bacteria.
- d. Incubate at 20 to 24°C for 24 hours to allow bacterial growth; all tests should be performed on 24 to 48 hour cultures.
- e. Using a sterile needle or small loop, pick individual distinct bacterial colonies representing each colony type. Use of a dissecting microscope can aid in distinguishing between differing colony types. Assign an isolate number to each isolated colony and record all colony characteristics on the data sheet.
- f. Begin initial identification of pure strain bacterial cultures (Section 2, 3.A1 Laboratory Reference Flow Chart Appendix 1).
 - i. Colony Pigmentation
Typical strains of *A. salmonicida* have brown diffusible pigment after 2 to 3 days of incubation. Some strains may not be pigmented (subsp. *achromogenes*).

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- ii. Gram Determination (Section 2, 3.8.A “Gram Reaction”)
A. salmonicida is Gram-negative. Gram-positive isolates may be reported as negative for *A. salmonicida*.
 - iii. Presence of Cytochrome Oxidase (CO) (Section 2, 3.8.B “Cytochrome oxidase”)
Most are CO positive. Rarely, CO negative strains of *A. salmonicida* subsp. *salmonicida* have been encountered (Chapman et al. 1991).
 - iv. Motility (Section 2, 3.8.C “Motility”)
A. salmonicida is non-motile. Motile isolates may be reported as negative for *A. salmonicida*.
- g. Perform biochemical testing on each isolate (Section 2, 3.A1 Laboratory Reference Flow Chart Appendix 1).
- i. Tube Method (Section 2, 3.8.D.1 “Tube Method”)
 - 1. Glucose fermentation (Section 2, 3.8.D.1.a “Glucose Fermentation”) using OF basal medium containing glucose will produce an oxidative/fermentative (O/F) or a fermentative (F) result with gas (most strains produce gas, but some may be weak or variable in this production). **Any isolate with a result other than this may be reported as negative for *A. salmonicida*.**
 - 2. Gelatinase (Section 2, 3.8.D.1.c “Gelatinase”) and Indole (Section 2, 3.8.D.1.d “Indole Test”) are considered together with the pigmentation of the isolate for interpretation of the results.
 - a. Brown diffusible pigmented isolates that are Gelatinase positive and Indole negative are **PRESUMPTIVELY positive** *A. salmonicida* sub-species *salmonicida*.
 - b. Non-pigmented isolates that are Gelatinase negative and Indole positive are **PRESUMPTIVELY positive** *A. salmonicida* sub-species *achromogens*.
 - 3. **Isolates yielding any other combination of these results may be reported as negative for *A. salmonicida*.**
 - 4. Additional biochemical tests will differentiate *A. salmonicida salmonicida* from *A. salmonicida achromogens*.
 - a. Maltose fermentation (Section 2, 3.8.D.1.e “Carbohydrate Utilization”) is determined using OF basal medium containing maltose, producing a fermentative F result. These tests may be done in a closed (i.e., overlaid) tube as is described for glucose (Section 2, 3.8.D.1.a “Glucose Fermentation”) or by interpreting the reaction at the bottom of an open tube that contains sufficient medium to distinguish fermentation from oxidation (e.g., minimum of 5 mL in a 16 x 25 mm tube). *A. salmonicida* sub-species *salmonicida* ferments maltose. *A. salmonicida* subspecies *achromogens* typically does not ferment maltose; however, maltose utilization has been documented for a few isolates.

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- b. Esculin hydrolysis (Section 2, 3.8D.1.h “Esculin Test”) is determined on Bile Esculin Agar slants with a resulting positive hydrolysis by *A. salmonicida* sub-species *salmonicida*, whereas strains of *A. salmonicida* sub-species *achromogens* are negative.
- ii. Commercial Identification Systems (Section 2, 3.8.D.2 “Commercial Identification Systems”)
 1. Biolog (Section 2, 3.8.D.2.b “Biolog”)
 2. API
If isolates are tested with the commercial system API described in Section 2, 3.8.D.2.a “API-20E,” it is recommended that the reference profiles be consulted in Section 2, 3.A2 Profiles Obtained with API-20E for Known Fish Pathogens.
- h. When testing is complete, either cryopreserve isolates of interest or discard bacterial plates and biochemical tubes in a biohazard bag and autoclave before proper disposal.
- i. Positive control isolates of *Aeromonas salmonicida* can be obtained from the American Type Culture Collection (ATCC). The Internet location for ATCC is <http://www.atcc.org>. Below are suggested isolates to use for positive control cultures:
 - i. *A. salmonicida* subspecies *salmonicida* – ATCC # 14174.
 - ii. *A. salmonicida* subspecies *achromogenes* – ATCC # 10801.
 - iii. A **cytochrome oxidase negative** isolate is also available – ATCC # 49385.

B. Confirmatory Test

1. **Fluorescent Antibody Test (FAT)** (Section 2, 3.8.E “Fluorescent Antibody Test (FAT)”)
FAT is performed on at least one representative isolate from each lot inspected and found positive during screening. Positive bacterial isolates will fluoresce strongly and have the same morphology as the positive control. A list of sources from which antibodies may be obtained is provided in Section 2, 3.8.E.6 “Commercial Sources for Antibodies.”