3.4 *Edwardsiella ictaluri* (Enteric Septicemia of Catfish, ESC)

The primary host species of this pathogen include all species of catfish and tilapia, as well as other warm water species. Both fingerlings and adults can be affected by the disease. In catfish, the bacterium is transmitted through the olfactory system to the brain, where the typical "hole-in-the-head" lesions can be observed during an *E. ictaluri* epizootic. Disease typically occurs at water temperatures between 20°C and 30°C. Experimental infections have been established in salmonids. It is generally accepted, that any warmwater species of fish can carry this pathogen with or without exhibiting signs of disease (Hawke et al 1981; Bullock and Herman 1985; Austin and Austin 1987; Thoesen 1994).

A. Summary of Screening Test

1. Bacterial Culture and Biochemical Analysis

a. Aseptically inoculate samples onto tubes or plates as described in Section 2, 2.2 Sampling.

b. Incubate for 24 to 48 hours at 28 to 30°C. Alternatively, this organism can be grown at 20 to 24°C (Plumb et al. 1989). If no growth occurs at 24 and 48 hours, record this information on the data sheet. **If no growth occurs after 96 hours, samples are discarded and reported as negative for *E. ictaluri*.**

c. When primary culture occurs on tubes or plates use a sterile loop or needle to select a single colony to subculture onto fresh TSA or BHIA plates. If colonies are not well isolated, a new plate will have to be streaked over the entire plate surface to achieve isolation of bacteria.

d. Incubate at the temperature used above 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 to represent each colony type. Use of a dissecting microscope can aid in distinguishing between differing colony types. Assign an isolate number to each pure 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. Gram Determination (Section 2, 3.8.A "Gram Reaction")
Edwardsiella ictaluri is Gram-negative. **Gram-positive isolates may be reported as negative for *E. ictaluri*.**

ii. Presence of Cytochrome Oxidase (CO) (Section 2, 3.8.B "Cytochrome Oxidase")
E. ictaluri is CO negative. **CO positive isolates may be reported negative for *E. ictaluri*.**

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. Triple Sugar Iron (TSI) (Section 2, 3.8.D.1.b "Triple Sugar Iron (TSI)")
E. ictaluri will yield an alkaline over acid (K/A) or alkaline over acid with gas (K/AG) result. **Any isolate with a result other than this may be reported as negative for *E. ictaluri*.**

2. Carbohydrate Utilization (Section 2, 3.8.D.1.e "Carbohydrate Utilization")
The following carbohydrates cannot be utilized (fermented) by *E. ictaluri*:

Arabinose
Rhamnose
Sucrose
Salicin

Isolates yielding positive results for any of these tests may be reported as negative for *E. ictaluri*.

3. Malonate Test (Section 2, 3.8.D.1.g "Malonate Test")
Isolates yielding positive results for this test may be reported as negative for *E. ictaluri*.

4. Indole Test (Section 2, 3.8.D.1.d "Indole Test")
Isolates yielding positive results for this test may be reported as negative for *E. ictaluri*.

5. Esculin Test (Section 2, 3.8.D.1.h "Esculin Test")
Isolates yielding positive results for this test may be reported as negative for *E. ictaluri*.

6. Decarboxylase Test (Lysine) (Section 2, 3.8.D.1.f "Decarboxylase Test (Lysine)")
Isolates yielding negative results for this test may be reported as negative for *E. ictaluri*.

7. Manitol Utilization Test (Section 2, 3.8.D.1.e "Carbohydrate Utilization")

a. **Isolates yielding positive results for this test may be reported as negative for *E. ictaluri*.**

b. Isolates yielding negative results for this test and that satisfy all previous conditions in this section are **PRESUMPTIVELY positive** for *E. ictaluri.*

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 Appendix 2."

3. 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.

4. Positive control isolates of *Edwardsiella ictaluri* can be obtained from the American Type Culture collection (ATCC). The Internet location for ATCC is http://www.atcc.org.

B. Summary of 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 inspection. 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."