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**INDUSTRY STANDARD OF THE  
PEOPLE'S REPUBLIC OF CHINA**

**SN/T 5188-2020**

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**Technical specifications for quarantine of Edwards sluggish  
disease**

迟缓爱德华氏菌病检疫技术规范

**(English Translation)**

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## Table of Contents

- Foreword
- 1 Scope
- 2 Normative references
- 3 Terms, definitions and abbreviations
- 4 Slow Edwards disease
- 5 Pathogen isolation and identification
  - 5.1 Instrument and equipment
  - 5.2 Medium
  - 5.3 Collection and treatment of disease materials
  - 5.4 Separation culture
  - 5.5 Staining microscopic examination
  - 5.6 Biochemical test
- 6 Edwards tardy nucleic acid test
  - 6.1 Reagents and materials
  - 6.2 Apparatus and equipment
  - 6.3 Template extraction
  - 6.4 Polymerase Chain Reaction (PCR)
  - 6.5 Real-time fluorescent PCR

### Foreword

This document is drafted in accordance with GB/T 1.1-2020.

### 1 Scope

This document stipulates the isolation and identification of Edwards sluggish bacteria, polymerase chain reaction (PCR) and real-time fluorescent PCR detection skills requirement.

This document is applicable to the detection of Edwardsiella slow in aquatic animals.

### 2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the

version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and test methods

### **3 Terms, definitions and abbreviations**

There are no terms and definitions that need to be defined in this document. The following abbreviations apply to this document.

E.tarda. Edwards tarda.

### **4 Slow Edwards disease**

Edwards sluggish disease is a general term for aquatic animal diseases caused by Edwards sluggish bacteria. Intestinal sepsis occurs mainly after fish infection Disease and liver and kidney necrosis. See Appendix A for information on the epidemiology, clinical symptoms, and pathological changes of Edwards sluggish disease.

#### **5.1 Instrument and equipment**

Microscope. 10x~100x.

#### **5.2 Medium**

##### **5.2.1**

Blood agar. see B.1.

##### **5.2.2**

Nutrient agar. see B.2.

##### **5.2.3**

Ferrous sulfate agar. see B.3.

##### **5.2.4**

Semi-solid agar. see B.4.

##### **5.2.5**

Sodium malonate medium. see B.5.

##### **5.2.6**

Peptone water. see B.6.

##### **5.2.7**

Glucose peptone culture medium. see B.7.

#### **5.2.8**

Lysine decarboxylase culture medium. see B.8.

#### **5.2.9**

ONPG medium. see B.9.

#### **5.2.10**

Sugar fermentation tube. see B.10.

#### **5.2.11**

Commercial media can be used.

### **5.3 Collection and treatment of disease materials**

Aseptically take an appropriate amount of liver, kidney and diseased tissues of diseased animals, place them in a sterile centrifuge tube, rinse with sterile saline, and homogenize for later use. Such as the test cannot be performed immediately, and the collected or processed samples must be stored at 4 °C ~ 8 °C, and no more than 24 hours.

### **5.4 Separation culture**

Take samples and homogenize them, inoculate blood agar plates with streaks, and incubate at 28 °C ± 1 °C for 24 h to 48 h. Pick out gray, round, moist, the translucent single colony was purified and cultured on a common nutrient agar plate.

### **5.5 Staining microscopic examination**

The suspicious bacteria were subjected to Gram stain and observed under a microscope. Edwards tarda is Gram-negative Brevibacterium, no capsule, no the spores are approximately (0.5 μm ~ 1 μm) x (1 μm ~ 3 μm) in size.

#### **5.6.1 Oxidase test**

Take the white clean filter paper and dip it into the colony, add a drop of 1% dimethyl p-phenylenediamine hydrochloride solution, the positive ones will appear pink, and gradually add Deep; add a drop of 1% alpha-naphthol solution, the positive person will appear bright blue within half a minute. The negative person does not change color within two minutes. Or mao the reagent is sucked by a thin pipette and directly dripped onto the colony. The color reaction is the same as the above.

#### **5.6.2 Biochemical identification test**

Suspicious bacteria with negative oxidase test shall be biochemically identified according to Appendix C.

#### **6.1.1 Water**

Should comply with GB/T 6682 Grade 1 water specifications.

#### **6.1.2 DNA extraction solution**

40 mmol/L Tris-HCl, pH8.0 20 mmol/L sodium acetate, 1 mmol/L EDTA, 1% SDS.

#### **6.1.3 10xPCR buffer**

Potassium chloride (KCl). 500 mmol/L; Tris HCl (pH 8.3). 100 mmol/L; gelatin. 0.1%; MgCl<sub>2</sub>.15 mmol/L.

#### **6.1.4 DNTPS**

dATP, dGTP, dTTP, dCTP each 2.5 mmol/L.

#### **6.1.5 Taq DNA Polymerase (5 U/μl)**

#### **6.1.6 50xTAE buffer**

Weigh 484 g Tris, measure 114.2 mL glacial acetic acid,.200 mL 0.5 mol/L EDTA (pH 8.0), dissolve in water, and dilute to 2 L.

After aliquoting, autoclave for later use.

### **6.2 Apparatus and equipment**

#### **6.2.1**

Thermal cycler.

#### **6.2.2**

Electrophoresis device.

#### **6.2.3**

Gel imaging system.

#### **6.2.4**

PCR ultra-clean workbench.

#### **6.2.5**

High-speed desktop centrifuge (speed above 12 000 r/min).

#### **6.2.6**

Bench-top centrifuge (speed above 2 000 r/min).