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

SN/T 5186-2020

Technical specifications for quarantine of abalone impetigo

鲍脓疱病检疫技术规范

(English Translation)

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Foreword

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

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organizations of this document. Haikou Customs of the People's Republic of China, Xiamen Customs of the People's Republic of China.

1 Scope

This document stipulates the clinical diagnosis, pathogen isolation, biochemical identification, PCR detection method and fluorescent PCR detection method of abalone impetigo.

This document is applicable to the pathogen detection of abalone impetigo.

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 4789.28 National Food Safety Standard Quality Requirements for Food Microbiological Inspection Medium and Reagents GB 19489 Laboratory Biosafety General Requirements GB/T 6682 Analytical laboratory water specifications and test methods GB/T 18088 Entry and Exit Animal Quarantine Sampling GB/T 19495.2 Technical requirements for genetically modified products testing laboratories SC/T 7103 Technical Specification for Quarantine and Sampling of Aquatic Animal Origin

3 Terms and definitions

There are no terms and definitions that need to be defined in this document.

4 Overview

It lasts for a long time and has a high mortality rate, causing extremely serious economic losses in Hainan Province and all over the country. *Vibrio fluvialis* is a halophilic bacterium, gram-stained Color negative, single flagella at the top, with power. This bacteria can produce enterotoxin and cytotoxin. This bacteria is sensitive to most broad-spectrum antibacterial drugs, but resistant Penicillin and cephalosporin antibiotics.

Abalones suffering from impetigo can see several slightly raised white pustules on the foot muscles. The shape of the pustule is basically triangular, and the lesion is from the lower surface of the gastropod gradually expands and penetrates into the interior of the foot. At the beginning, the lesion was small, and as the course of the disease progressed, the lesion gradually expanded.

Refer to Appendix A for clinical quarantine requirements.

5.1 Reagents

5.1.1

Unless otherwise specified, all chemical reagents required for the test should be of analytical grade.

5.1.2

The test water should meet the first-grade water specifications in GB/T 6682, and the test water used for DNA extraction and PCR test should be consumed.

Bacteria distilled water.

5.1.3

Culture medium.

5.1.4

Biochemical identification reagents. arginine dihydrogenase, lysine deshydrogenase, ornithine deshydrogenase, urease, sugar fermentation tube, trace biochemical Tube, biochemical identification reagent strip or kit, etc.

5.1.5

Bacterial genome extraction kit.

5.1.8

TBE running buffer.

5.1.9

Nucleic acid stains.

5.2 Equipment

5.2.1

Refrigerator.

5.2.2

Incubator.

5.2.3

Ordinary PCR machine.

5.2.4

High-speed refrigerated centrifuge.

5.2.5

Balance.

5.2.6

Fluorescent PCR machine.

5.2.7

Biological safety cabinet.

5.2.8

Electrophoresis instrument.

6.1 Sampling

The live abalone is dissected, and the tissues and organs such as foot, mantle, gonad, hepatopancreas, digestive tract, etc. are taken out and ground for use. Number of samples, Packaging, etc. can be implemented in accordance with GB/T 18088 or SC/T 7 103.If the

importing country or region has specific requirements, press the importing country or region sampling.

6.2.1 Enrichment

Biosafety should comply with the relevant regulations of GB 19489.

Add the collected abalone tissue to alkaline peptone water (see B.1) containing 3% NaCl, and incubate at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ 8 h~18 h.

6.2.2 Vaccination

Use an inoculating loop to inoculate the culture solution on the TCBS medium (see B.2), and incubate at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18 h to 24 h.

6.2.3 Pure culture

Select a single yellow colony to inoculate the nutrient broth medium (see B.3), and culture it at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18 h to 24 h.

6.2.4.1 Plate culture characteristics

After culturing at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 18 h to 24 h, *Vibrio fluvialis* will appear yellow colonies on TCBS.

6.2.4.2 Gram stain

After selecting the pure culture, perform gram staining on the suspicious colonies according to GB 4789.28. *Vibrio fluvialis* is a gram-negative bacterium.

6.2.5 Biochemical identification

The strains preliminarily identified as *Vibrio fluvialis* by 6.2.4 shall adopt an automatic biochemical identification system or be inoculated with arginine dihydrogenase, lysine and arginine dihydrogenase.

Bacterial microbiochemical identification tube for amino acid deshydrogenase, ornithine deshydrogenase, urease, sugar fermentation tube, oxidase, indole, etc., set at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ Cultivate for 48 h, and judge according to the instructions of the bacterial microbiochemical identification tube.

Lysine deshydrogenase, ornithine deshydrogenase, urease and VP tests are all negative; lactose does not ferment, does not produce H₂S, and glucose produces acid no gas is produced; samples that are both positive for arginine dihydrogenase and oxidase are judged to be positive for *Vibrio fluvialis*.

See Appendix C for the main biochemical identification results of *Vibrio fluvialis*.