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

SN/T 5189-2020

**Technical specifications for quarantine of catfish intestinal
sepsis**

鮰类肠败血症检疫技术规范

(English Translation)

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Foreword

This document is drafted in accordance with the rules given in 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. Fuzhou Customs of the People's Republic of China, Xiamen Customs of the People's Republic of China, Fuzhou Disease Control and Prevention center.

1 Scope

This document specifies the clinical diagnosis, bacterial isolation, biochemical identification and polymerase chain reaction identification technology for catfish intestinal sepsis.

This document is suitable for clinical diagnosis and laboratory quarantine of catfish intestinal sepsis.

2 Normative references

The contents of the following documents constitute an indispensable clause 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 SC/T 7014
Technical specification for aquatic animal quarantine experiment SC/T 7103 Technical
Specification for Quarantine and Sampling of Aquatic Animal Origin SC/T 7214 Method for
detection of Edwardsiella in fish

3 Terms and definitions

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

4.1 Group inspection

Diseased fish ponds are often of poor water quality. The diseased fish swim alone along the edge of the pond, are slow to respond, decrease their food intake, or do not eat. In the early stage of illness, the disease Fish pectoral fins often have damages with a diameter of 3 mm to 5 mm, such as needle-like wounds on the outside, and penetrate deep into the muscles. Some sick fish have their heads up and their tails up the bottom is swimming vertically, and some diseased fish sometimes swim in a convulsive spiral shape, and then die.

4.2 Individual examination

Due to differences in catfish specifications and individual immunity, the clinical symptoms of catfish intestinal sepsis are not the same, mainly showing acute and chronic Type in two forms.

Acute type, also known as intestinal sepsis type, is the most common type with high mortality, mainly manifested as sepsis and enteritis. Fading on the surface of sick fish Spots, swelling of the abdomen, some skin ulcers on the head and body, prominent eyeballs on one or both sides, and a bit of bleeding or bleeding spots on the muscles. Gill silk green White with bleeding spots, ecchymosis and bleeding on the skin of the operculum, abdomen, jaw and fin base, while some have white spots, red and swollen anus, in the later stage, the diseased fish showed generalized edema.

The chronic type is also known as the cranial perforation type. The initial infection site of the bacteria is the olfactory sac of the root of the nose, and then migrate to the brain through the olfactory organs to form flesh.

Swelling inflammation. Later, the side of the head and dorsal skull ulcerated to form a deep hole, until the entire brain tissue was exposed, forming a "saddle"-like lesion;

As the disease progresses, ulcers form in the center of the lesion, which manifests as a "cephalic perforation type" disease.

4.3 Anatomy

Open the abdominal cavity of the diseased fish, there is a lot of blood or clear fluid in it, and the ascites flowing out is not easy to coagulate. Liver edema, brittle texture, bleeding spots and Gray-white necrosis; spleen and kidney swelling and bleeding; gastric enlargement; intestinal dilatation, intestinal mucosa edema, congestion, inflammation, intestinal cavity full of gas Body or pale yellow watery liquid; sometimes accompanied by the appearance of the intestine. The brain tissue forms granulomas, and the muscles have diffuse granulomatous inflammation.

4.4 Judgment by clinical examination

The above symptoms appear in the group examination and individual examination, combined with the results of the necropsy, it can be preliminarily judged as suspected catfish intestinal sepsis.

Further laboratory diagnosis.

5.1 Equipment

5.1.1

Optical microscope.

5.1.2

Incubator ($28\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$).

5.1.3

Water bath.

5.1.4

High-speed centrifuge.

5.1.5

Micro adjustable pipette.

5.1.6

Thermal cycler.

5.1.7

Electrophoresis instrument.

5.1.8

Ultraviolet gel imaging system.

5.1.9

Alcohol lamp.

5.2 Materials and reagents

5.2.1

Water should meet the requirements of Grade 1 water specified in GB/T 6682.

5.2.2

For blood agar plates, see Appendix A.1 for the preparation method.

5.2.3

Lu's alkaline methylene blue dye solution, the preparation method is shown in Appendix A.2.

5.2.4

Crystal Violet Staining Solution, the preparation method is shown in Appendix A.3.

5.2.5

Gram iodine solution, the preparation method is shown in Appendix A.4.

5.2.6

Safranine counterstain solution, the preparation method is shown in Appendix A.5.

5.2.7

Semi-solid agar tube. See Appendix A.6 for the preparation method.

5.2.8

Tetramethyl-p-phenylenediamine 1% aqueous solution, the preparation method is shown in Appendix A.7.

5.2.9

Hugh-Leifson medium. See Appendix A.8 for the preparation method.

5.2.10

Phosphate buffered saline (PBS), see Appendix A.9 for the preparation method.

5.2.11

The preparation method of TE buffer (pH 8.0) is shown in Appendix A.10.