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**Detection of diphyllbothroid larvae
(English Translation)**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

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Split head tapeworm larva detection

1 Scope

This standard specifies the operating procedures for the detection of larvae of the larvae.

This standard applies to disease prevention and control institutions at all levels, medical institutions, and food testing institutions for the inspection of larvae of *Schistocephalus* in fish, frogs and snakes.

Measurement.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this document.

For undated references, the latest version (including all amendments) applies to this document.

GB/T 18088 Entry and Exit Animal Quarantine Sampling Standard GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and definitions

The following terms and definitions apply to this document.

Sparganosis

The larvae of *Schizocephalus* are the third stage larvae of *Pseudophylla* and *Schizocephae* the general term for insects (see Appendix A).

Sparganosis

The third-stage larva of *Schizocephalus* parasitic in fish.

Sparganosis *mansoni*

The third-stage larva of *Dimonia mansoni* parasitic in frogs, snakes, or humans.

4 Equipment

4.1

Biological microscope.

4.2

Stereo microscope.

4.3

PCR thermal cycler.

4.4

Electrophoresis instrument.

4.5

Gel imaging system.

4.6

Ultra-clean workbench.

4.7

High-speed centrifuge.

4.8

Surgical instruments for dissection.

5.1 Reagents

Pepsin digestion solution; Tris-acetic acid electrophoresis buffer (TAE); agarose gel; 6x loading buffer; 100~2000 bp DNA marker; Sterilized double distilled water (ddH₂O); 70% ethanol (see Appendix B, B.1 for preparation).

5.2 Primer

Amplification of the primer sequence of the schistocyst ribosomal DNA transcription spacer (ITS); amplification of the primer sequence of the cytochrome C oxidase (Cox1) of the schistocyst Columns; the sequence of the specific primers of *S. mansoni* and the sequence of the specific primers of *S. mansoni* (see Appendix B, B.2).

5.3 Positive control

The positive control is the positive clone plasmid of the corresponding gene fragment of Schizoccephalus or the whole genome DNA of Schizoccephala and Schizoccephalus.

6.1 Sample preparation

6.1.1

Types of samples. marine fish, freshwater fish, frogs and snakes.

6.1.2

Sample collection. Sampling shall be carried out in accordance with GB/T 18088.

6.2.1 Tablet inspection method

Use surgical scissors to dissect the examined fish, frogs and snakes one by one. Take out the internal organs, scrape off the inner lining of the abdominal cavity and observe the surface of the abdominal cavity. If there are suspicious white spots on the surface, use surgical scissors or a scalpel to separate the skin and flesh, use two small tweezers to tear open the muscles, and take out the white spots.

The tissues were pressed with glass slides, and the results were determined by microscopic examination with a biological microscope.

6.2.2 Protease digestion method

Weigh 250 g of the sample and cut it into small pieces, add the digestion solution at a ratio of 1.5 between the sample and the pepsin digestion solution, and digest at 37°C until there is no naked eye Visible fleshy tissue. After digestion, filter with a 0.8mmx0.8mm (10 mesh) mesh sieve, and place the filtrate in a sharp-bottomed graduated cylinder and add water (with water According to GB/T 66828) to the maximum scale, the sediment is washed until the water is clear, all the sediment is placed in a flat dish, and the result is determined by a stereo microscope.

6.2.3.1 Sampling

Pick the worms under a stereo microscope and use them for preliminary identification. Set up a positive control for PCR reaction, the control is the corresponding gene sheet of Schizocephalus the positive cloning plasmid or the whole genomic DNA of schistocephala and schistocephala. Sterile water was used as a blank control.

6.2.3.3 Reaction system

The total volume is 25 L, template DNA 2 L, upstream and downstream primers (10 mol/L) each 0.5 L, dNTPs 2 L, MgCl₂ 2.5 L, 10xBuffer 2.5 L, Taq enzyme (5 U/μL) 0.2 L, supplement with ddH₂O to 25 L.

6.2.3.4 PCR reaction program

Pre-denaturation at 94°C for 3 min; denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, 35 cycles; extension at 72°C for 7 min.

6.2.3.5 Electrophoresis

Take 10 L of product and 2 L of 6x loading buffer to mix, add sample to 1.5% agarose gel containing ethidium bromide. In 1xTAE in the buffer, electrophoresis at 3 V/cm~4 V/cm for about 30 minutes. When the bromophenol blue reaches the bottom, the electrophoresis is stopped, and the gel imaging system is used for analysis.

6.3.1 Schizocephaly identification

If the size of the larva detected from the fish is 2mm~20 mm long, 2mm~3 mm wide, milky white, the head is spoon-shaped, and its dorsal and ventral surfaces are different There is a narrow and deep concave suction groove, the front end of the body is concave and slightly larger, the body is not segmented but has horizontal folds, and the tail is thin, stick-like, with and the cephaloceles similar to worms can be preliminarily identified as schistocephala of the genus Schizocephalus (see Appendix C).