

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

SN/T 5109-2019

**Method for detection of Yersinia tyrosin polymerase spiral
constant temperature amplification in border ports**

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(English Translation)

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Foreword

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

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

This standard was drafted. China Academy of Inspection and Quarantine, Manzhouli Customs of the People's Republic of China, Fuzhou Sea of the People's Republic of China Guan, Chinese People's Liberation Army Institute for Disease Control and Prevention.

1 Scope

This standard specifies the method for detecting *Yersinia pestis* polymerase spiral thermostated amplification (PSR) at border crossings, including sample preparation, testing procedures Procedure, method, result judgment and report.

This standard is applicable to the screening of *Yersinia pestis* samples from border ports at suspected infections of *Yersinia pestis*, rodents, and fleas.

Detection.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this article. For undated references, the latest version (including all amendments) applies to this document.

GB 1849 General requirements for laboratory biosafety WS233 General Guidelines for Biosafety in Microbiology and Biomedical Laboratories List of human pathogenic microorganisms (Ministry of Health of the People's Republic of China)

3 Terms and definitions

The following terms and definitions apply to this document.

The taxonomy is protozoa, prokaryotic type microorganisms, gram-negative facultative anaerobic bacteria, Enterobacteriaceae, Yersinia, pseudoknots. Pneumonic plague is one of the important pathogens of biological warfare agents and biological terror. F1 antigen is one of the recognized Y. pestis-specific antigens.

The F1 gene is often used as a target for nucleic acid detection of Yersinia pestis.

A method for isothermal nucleic acid amplification, which uses 2 to 4 primers to specifically recognize 4 to 6 independent regions of a target fragment.

With the significant decrease in pH, the pH indicator can be added to determine the result by observing the color change; fluorescent dyes can also be added (for example.

EveGreen), the results were judged by the amplification curve and dissolution curve on a fluorescence PCR instrument.

4 Abbreviations

The following abbreviations apply to this document.

PSR. Polymerase Helix Isothermal Amplification Reaction (polymerase isothermal amplification)
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DNA. deoxyribonucleic acid dNTP. deoxyribonucleoside triphosphate (deoxy ~ ribonucleoside triphosphate)

5 Detection object

Detection objects include.

--- Unknown samples and environmental samples appearing at border crossings;

--- Samples of rodents, parasitic fleas, dry holes, etc .;

--- Symptoms of people at the border crossing from the plague area or other people who cannot exclude *Yersinia pestis* infection.

6 Biosafety requirements

The biosafety requirements during the experiment are as follows.

--- Personal protection should be done in accordance with the relevant requirements in GB 1849 and WS 233 during testing;

--- It should be carried out in accordance with the biosafety requirements in the "List of Pathogenic Microorganisms Infected by Humans".

Level 2 (BSL-2) laboratory;

--- The waste products shall be treated in accordance with GB 1849's requirements for waste treatment.

7.1 Main instruments

The main equipment is as follows.

Fluorescence PCR instrument; or nucleic acid amplification instrument; or constant temperature amplification detection instrument; or metal bath; or water bath. $65\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$; $95\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

7.2 Main reagents

The main reagents used are as follows.

--- Multi-function sample processing solution. 5% Celex-100, 1% TritonX-100 TE buffer (Tris 10MM, EDTA 1mm) (see Appendix A for specific preparation methods);

--- Rapid constant temperature amplification kit for *Yersinia pestis*. Including nucleic acid-free extraction reagents (multifunctional sample processing tube 1, containing multifunctional samples Product treatment solution), *Yersinia pestis* one-drop on-site detection kit 2 (see Appendix B for specific methods), *Yersinia pestis* real-time fluorescence PSR Test Kit 3 (see Appendix C for specific methods);

Note 1,2,3. Reagents 1, 2, and 3 are provided by the designated unit. This information is provided for the convenience of users of this standard and does not indicate approval of the product. in case Other equivalent products have the same effect, you can use these equivalent products.

--- Primer. A primer set designed based on the conserved sequence of the F1 gene of *Yersinia pestis* (GenBank number. X619.96.1) is composed as follows.

FP. AAGGTACCATTTGGGATTCACCCCCGCGCGCTATAAAAACAGGAAC BP.
AAGTACATACTGGGATCACCCCCGCCCCACATACCTCTAGCTACTGCTCCTGATCA IF.

GCATTCGTAGAAGTAGAAGTAGTCGC

--- dNTPs. four nucleotides, each at a concentration of 10 mm;

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--- Reaction buffer (RM). Tris-HCl 20.0 mm, ammonium sulfate 10.0 mm, potassium chloride 10.0 mm, magnesium sulfate 8.0 mm, Tween- 20.1%, betaine 0.8M, dNTP (each) 1.4mm, primers FP, BP each 1.6μM, IF0.8μM, pH 8.8;

--- Color development solution. cresol red 2.0mm, phenol red 0.5mm. Or fluorescent dye. EverGreen (20 x aqueous solution). Choose either

--- Positive control. traceable identified strain, or DNA containing the target fragment.

8.1.1 Environmental samples such as white powder and surface of objects

Wet a sterile cotton swab with sterile physiological saline or multi-functional sample treatment solution, soak white powder, or wipe the surface of the contaminated object.

In a test tube containing 2mL of multifunctional sample processing solution, the oil-based pen number is marked for inspection.

8.1.2 Samples of rodent organs

Tissues such as liver, spleen, lung, heart blood, and lymph nodes with suspicious pathological changes, which are collected by aseptic technique, are added in 4 to 8 drops (about 100 μL to 200 μL) grinding solution (normal saline or multi-functional sample processing solution) grinding. Use a sampling cotton swab to dip the ground homogenate (about 50 μL ~ 100μL), placed in a test tube containing 2mL of multi-functional sample processing solution, oily pen number marked for inspection.

8.1.3 Flea samples

After the species are identified, they are grouped by collection time, place, and type, and 10 to 20 are a group of samples to be tested. Sample to be added 4 to 8 drops (about 100 μL to 200 μL) of grinding liquid (physiological saline or multifunctional sample processing solution) are ground. Use a sampling cotton swab to dip the ground homogenate (about 50 μL to 100 μL) and place it in a test tube containing 2 mL of multifunctional sample processing solution. The oily pen number is marked for inspection.

8.1.4 Human samples

May include human venous blood, lymph fluid, bloody sputum, bloody stools, pharyngeal secretions, eye secretions, blisters/pustules, cerebrospinal fluid, ulcers and wounds under the skin. Aseptically collected venous blood from 3 to 5 mL of the subject, and allowed to stand at room temperature for several minutes to coagulate the blood. The upper layer is