

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

SN/T 5107-2019

**Method for detecting multi-pathogen gene suspension chip of
mosquito-borne infectious diseases at entry and exit ports**

出入境口岸蚊媒传染病多种病原体基因悬浮芯片检测方法

(English Translation)

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Table of Contents

Foreword	
1 Scope	
2 Normative references	
3 Terms and definitions	
4 Biosafety protection requirements	
5 Object	
6 Collection, storage and transportation of samples	
6.1 Sample collection	
6.2 Submission and processing of samples	
7 Major equipment and reagents	
7.1 Major equipment	
7.2 Main reagents	
8 Detection method	
8.1 DNA extraction	
8.2 RNA extraction	
8.3 PCR amplification	
8.4 Coupling of probes with carboxylated microspheres	
8.5 Crossing	
8.6 Determination of fluorescence value	
8.7 Determination of results	

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. Shijiazhuang Customs, People's Republic of China.

1 Scope

This standard provides for rapid screening of yellow fever virus, chikungunya virus, West Nile virus, Japanese encephalitis virus, dengue Virus (type I ~ IV), Plasmodium (Plasmodium vivax, Plasmodium falciparum, Plasmodium falciparum, Plasmodium ovale) 6

types of mosquito vectors of special interest Genetic Suspension Chip Screening Methods for Pathogens.

This standard is applicable to screening of entry and exit personnel or mosquitoes carrying the above six pathogens at frontier ports.

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

GB 1849 General requirements for laboratory biosafety SN/T 2878 Requirements for the storage and transportation of medical vector samples WS233 General Guidelines for Biosafety in Microbiology and Biomedical Laboratories

3 Terms and definitions

The following terms and definitions apply to this document.

It is a natural epidemic disease transmitted by vector mosquitoes. The common diseases include Japanese encephalitis, malaria, dengue fever, filariasis and yellow fever.

Strong infectious disease.

A biochip for large-scale determination of nucleic acids by using encoded microspheres as a carrier and flow cytometry as a detection platform technology.

4 Biosafety protection requirements

4.1

The laboratory shall follow the biosafety requirements of GB 1889 and WS233 for biosafety class II (BSL-2) laboratories.

4.2

The used experimental supplies shall comply with the requirements of GB 1849, and the waste shall be treated harmlessly.

5 Object

5.1

Samples of suspicious cases intercepted at ports of entry and exit from personnel from countries or regions where mosquito-borne diseases are endemic.

5.2

Mosquito samples were intercepted at entry and exit ports from countries or regions where mosquito-borne infectious diseases are endemic.

SN/T 5107-201919 ?????? ?????, ?????, ??

6.1 Sample collection

6.1.1

Collect blood from people in the epidemic area, suspicious entry-exit personnel, and applicants for inspection.

6.1.2

Collect samples of mosquitoes or other means of mosquitoes concealed from vehicles or transportation equipment from infected areas.

6.2 Submission and processing of samples

6.2.1

Mosquito samples. Put the collected mosquitoes directly into the -20 °C refrigerator to freeze to death, take them out, sort them by number, and pack 30 ml into 2ml Inside the screw mouth plastic tube, transport or store at -70 ° C for inspection.

6.2.2

Serum and plasma. Absorb directly into a sterile centrifuge tube with a sterile syringe for processing, and number it for future use.

7.1 Major equipment

Suspension chip system, fluorescence microscope, level electrophoresis system, gel imaging system, centrifuge, PCR instrument, biological safety cabinet, pipette, nuclear Acid protein detector, vacuum pump, vortex shaker.

7.2 Main reagents

MEM and Hank's solution, specimen treatment solution (see A.1 in Appendix A), PCR reaction solution (see A.2 in Appendix A), tetramethylammonium chloride (TMAC), 1.5 x TMAC hybridization solution (see A.3 in Appendix A), 1 x TMAC hybridization solution (see A.4 in Appendix A), carboxylation Code microspheres (BioRad), 2 [N-morpholine] ethanesulfonic acid (MES) (pH 4.5), 1- (3-dimethylaminopropyl) -3-ethylcarbodiimide hydrochloride Salt (EDC), SDS, N-dodecyl sarcosinate, TE (pH 8.0) (see Appendix A.5), streptavidin-phycoerythrin (SA-PE), beta Diamine tetraacetic acid (EDTA), 75% ethanol, Trizol, chloroform, Tween-20, random primers, AMV reverse transcription kit, 20% chelation Resin Cherex100.

8.1.1 Method for extracting Plasmodium DNA from blood sample

8.1.1.1

Take 20µL of whole blood and place it in a 1.5mL centrifuge tube.

8.1.1.2

Add 200 µL preheated 5% Cellex 100, heat at 100 ° C for 10 minutes, and shake 3 to 5 times during this period.

8.1.1.3

Centrifuge at 12,000 g for 3 min. Take the supernatant to a clean 0.5 mL centrifuge tube and store at 4 ° C or -20 ° C until use.

8.1.2 Method for extracting Plasmodium DNA from mosquito samples

8.1.2.1

Grind the mosquito samples to be inspected in a tissue grinder or homogenize in an electric homogenizer for 20s to 30s to homogenize the samples into a paste.

Centrifuge at 2,000 g for 3 min, and take the supernatant as a sample.

8.1.2.2

Add 150 µL of re-distilled water and 50 µL of 20% Cellex 100 and heat for 10 min at 95 ° C to extract DNA.

8.1.2.3

During the heating process, vortex every 2 min.

8.1.2.4

Centrifuge the mixture at 2,000 g for 3 min. Take the supernatant into a clean 0.5 mL centrifuge tube and store at 4 ° C or -20 ° C.

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SN/T 5107-201919 ?????, ?????, ??

8.2 RNA extraction

8.2.1

Remove the mosquito sample to be tested from the container at -70 ° C, operate on ice, pour it into the grinder, and add 1 mL of Hank's solution to rinse it.

After discarding the liquid, add 1 mL of the sample treatment solution, and repeatedly grind until the tissue fragments have basically disappeared, and then suck the abrasive liquid into 1.5 mL Eppendorf centrifuge tube, equilibrated and centrifuged at 4 ° C and centrifuged