

ICS 65.020.30
CCS B 41

SN

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

SN/T 5336-2020

**Swine fever virus and African swine fever virus detection
microfluidic chip method**

猪瘟病毒及非洲猪瘟病毒检测 微流控芯片法
(English Translation)

Issued on: December 30, 2020

Implemented on: July 1, 2021

Issued by: General Administration of Customs

Table of Contents

Foreword
1 Scope
2 Normative references
3 Terms, definitions and abbreviations
3.1 Terms and definitions
3.2 Abbreviations
4 Principle
5 Reagents and materials
6 Equipment and equipment
7 Biosecurity measures
8 Test method
8.1 Sample
8.2 Nucleic acid extraction

Foreword

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

1 Scope

This document specifies the microfluidic chip detection method for swine fever virus and African swine fever virus.

This document applies to the detection of swine fever virus and African swine fever virus. For suspected swine fever viruses that cannot be distinguished by clinical symptoms Samples from cases of European swine fever virus infection can be identified and tested by the methods specified in this standard.

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/T 6682 Analytical laboratory water specifications and test methods GB/T 16551
Diagnosis technology of swine fever GB/T 18648 African swine fever diagnostic technology

GB 19489 Laboratory Biosafety General Requirements GB/T 27401 Laboratory Quality Control Specification Animal Quarantine

3.1 Terms and definitions

The following terms and definitions apply to this document.

3.1.1

Microfluidics

Microfluidics is the science and technology involved in systems that use micropipes (tens to hundreds of microns in size) to process or manipulate tiny fluids.

It is a new interdisciplinary subject involving chemistry, fluid physics, microelectronics, new materials, biology and biomedical engineering.

3.1.2

Microfluidic chip technology Integrate basic operation units such as sample preparation, reaction, separation, and detection in the biological, chemical, and medical analysis process into a micro A technology that automatically completes the entire analysis process on a meter-size chip.

3.2 Abbreviations

The following abbreviations apply to this document.

4 Principle

Nucleic acid sequence design for the 5' non-coding region of swine fever virus (CSFV) and the VP72 gene of African swine fever virus (ASFV) Primers, and fix them on the corresponding positions of the microfluidic chip, then encapsulate the microfluidic chip, and combine the extracted nucleic acid template with the reverse After mixing the reaction solution (containing SYBR Green fluorescent dye), add it to the packaged microfluidic chip, and then put it into the centrifugal function, in a microfluidic chip detector that integrates constant temperature function and real-time fluorescence detection, centrifugal force is used to drive the sample into the microfluidic chip for reaction Well for reverse transcription and constant temperature amplification. If the sample contains the target fragment and is amplified at a constant temperature, the amplified product will bind with the fluorescent substance.

Together, the fluorescence signal is captured in real time by the fluorescence detector, and the production of the amplified product is intuitively reflected. According to the appearance time of the real-time fluorescence signal, Strength and location to determine whether the sample contains CSFV, ASFV.

5 Reagents and materials

5.1

See Appendix A for reagent components and storage conditions. Unless otherwise specified, the reagents used in the test are of analytical grade, and laboratory water is used should meet the requirements of GB/T 6682. All reagents are aliquoted in containers without nuclease contamination.

5.2

The components of the negative control, positive control and internal reference control are as follows.

- a) Negative control. RNase-free water;
- b) Positive control. nucleic acid extracted from a positive sample, or artificially synthesized DNA fragment containing the target nucleic acid sequence;
- c) Internal reference control. including primers for amplification of internal reference gene fragments (non-amplified CSFV and ASFV nucleic acid fragments) and artificially synthesized the internal reference plasmid (non-ASFV and CSFV gene fragments), in which the primers are fixed in the reaction wells of the microfluidic chip, and the internal reference plasmid exists in in the reaction solution.

5.3

Reaction solution. 20 mmol/L Tris-HCl (pH 8.8), 10 mmol/L KCl, 10 mmol/L (NH₄)₂SO₄, 8 mmol/L MgSO₄, 0.1% Tween-20, 1.4 mmol/L dNTPs, 8000 U/mL Bst DNA polymerase, 0.5 mg/mL FEN, 50 μmol/L SYBR Green fluorescent stain Material, internal reference plasmid (400 copies/μL).

5.4

Nucleic acid extraction reagents. DNA and RNA can be extracted at the same time, including protease (20 mg/mL), magnetic beads (50 mg/mL), lysis solution, Isopropanol, lotion 1, lotion 2, lotion 3 and eluent. See Appendix B for the reagent preparation method. Other equivalent nucleic acid extractions can also be used Reagents or reagents for separately extracting DNA or RNA.

5.5

High temperature reverse transcriptase. 5000 U/mL.

5.6

RNase inhibitor. 40 U/μL.

5.7

Primer. See Appendix A for the primers of swine fever virus and African swine fever virus.

5.8

Microfluidic chip. 5 μL /reaction cell, 8x4 centrifugal microfluidic chip. Refer to Appendix C for the production of microfluidic chips, or according to the actual demand chooses other types of equivalent commercial microfluidic chips.

5.9

Consumables. Parafilm, RNase-free tip (10 μL , 100 μL , 1000 μL), RNase-free centrifuge tube (1.5 mL, 2 mL).

6 Equipment and equipment

6.1

Microfluidic chip detector.

6.2

Pure water meter.

6.3

Constant temperature drying oven.

6.4

Vortex oscillator.

6.5

Micro spotting instrument.

6.6

Low-temperature refrigerated centrifuge.

6.7

Low-temperature refrigerator.

6.8

Pipettes (0.5 μL , 10 μL , 100 μL , 1000 μL).

7 Biosecurity measures

7.1

Laboratory equipment, facilities and waste disposal requirements should meet the requirements of GB 19489.