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

SN/T 5207-2020

Quarantine and identification method of blueberry shock virus

蓝莓休克病毒检疫鉴定方法

(English Translation)

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Foreword

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

1 Scope

This document stipulates the principles of blueberry shock virus quarantine and identification, equipment, appliances and reagents, detection and identification, and determination of results and records, as well as the preservation of samples.

This document is applicable to the detection of blueberry shock virus in blueberry seedlings in and out of the country.

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.

SN/T 2122 Quarantine sampling method for entry and exit plants and plant products

3 Terms and definitions

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

4 Basic information

The basic information of blueberry shock virus is as follows.

--Scientific name. Blueberry shock virus, abbreviation. BISHV.

--Classification status. member of Bromoviridae and Ilarvirus.

--Classification status, host range, disease symptoms, distribution area, transmission route, morphology of blueberry shock virus, virus See Appendix A for genomes, etc.

5 Principle

The serological and molecular biological characteristics of blueberry shock virus are the main basis for formulating this quarantine identification method.

6.1 Equipment

High-speed refrigerated centrifuge, electronic balance (1/1000 g), PCR machine, electrophoresis instrument, horizontal electrophoresis tank, -20°C low-temperature refrigerator and -80°C ultra-low temperature refrigerator, automatic enzyme-linked immunoassay system, gel imaging analysis system, pH meter, microwave oven, magnetic stirrer Stirrer, constant temperature water bath, autoclave, ultra-clean workbench, microplate reader, etc.

6.2 Utensils

Adjustable pipettes of various volumes (1 000 µL, 200 µL, 100 µL, 20 µL, 10 µL, 2 µL), PCR reaction tubes, RNase-free centrifuge tube (1.5 mL), mortar, enzyme-linked plate, etc.

6.3 Reagents

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) reagents are shown in Appendix B, and common RT-PCR detection reagents are shown in Appendix C.

See Appendix D for nested RT-PCR detection reagents, and Appendix E for real-time fluorescent RT-PCR detection reagents.

7.1 Sampling inspection

Carry out sampling and sampling according to the method given in SN/T 2122, and check whether the plants are dwarfed, deformed, leaf mosaics, spots Barge and other suspected symptoms of virus harm.

7.2 DAS-ELISA

See Appendix B for the detection method.

7.3 Ordinary RT-PCR detection

See Appendix C for the detection method.

7.4 Nested RT-PCR detection

See Appendix D for the detection method.

7.5 Real-time fluorescent RT-PCR detection

See Appendix E for the detection method.

8.1 Judgment of results

8.1.1

DAS-ELISA preliminary screening, if the test result is negative, it is determined that the sample does not carry BISHV; if the test result is positive, Use any method of ordinary RT-PCR detection, nested RT-PCR detection or real-time fluorescent RT-PCR detection for verification.

8.1.2

If the verification result is positive, it is determined that the sample carries BISHV.

8.2 Record of results

Record various experimental data, including sample source, type, time, experimental time, location, method and results, etc., and There must be the signatures of the handlers and experimenters. Serological test results retain the data report of the absorbance value, and

the molecular biology test results are guaranteed Keep electrophoresis photos.

9 Storage of samples

The samples that are judged to be positive by the test result should be properly stored in an ultra-low temperature refrigerator at -20°C~-80°C, and be registered and marked.

Work in preparation for review.

Appendix A

(Informative)

Background information on blueberry shock virus A.1 Classification status Blueberry shock virus is a member of Bromoviridae and Ilarvirus.

A.2 Host range

The reported natural host is blueberry (*Vaccinium* spp.).

A.3 Disease symptoms the main symptom is the sudden and complete necrosis of flowers and leaves during the flowering period, resulting in no fruit. According to statistics, the virus infects blueberries caused by the loss can reach 34%~90%. BISHV can infect almost all blueberry varieties and show similar symptoms. Since BISHV can communicate Since bees carry infected pollen to spread, it spreads quickly in the field and easily occurs in a large area. Blshv caused by blueberry infection the symptoms are shown in Figure A.1.

A.4 Distribution area Mainly distributed in the United States, Canada and other countries.

A.5 Transmission route it is spread by bees carrying infected pollen, and it can also be spread over long distances through virulent seedlings.

A.6 Chondrial morphology the virions are equiaxially symmetrical and spherical with a diameter of about 27 nm.

A.7 Viral genome

Sense single-stranded RNA virus.

Appendix B

(Normative)

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) B.1

Reagents B.1.1 Coating antibody Specific blueberry shock virus antibody.

B.1.2 Enzyme-labeled antibody Alkaline phosphatase labeled blueberry shock virus antibody.

B.1.3 Substrate