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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 3449-2012

Detection and identification of candidatus phytoplasma in insect vector

昆虫介体中植原体检疫鉴定方法

(English Translation)

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Foreword

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

This standard is proposed and managed by the National Certification and Accreditation Administration Committee.

This standard was drafted. China Inspection and Quarantine Science Research Institute, People's Republic of China Ningxia Exit Inspection and Quarantine.

1 Scope

This standard specifies the quarantine and identification of insect vectors of phytoplasma.

This standard applies to the entry and exit quarantine and domestic monitoring Homoptera and Hemiptera (with sucking) insects and phytoplasma Identification.

Principle 2

Molecular biological characteristics (see Appendix A) and phytoplasma were identified based on the type of insect vectors spread of phytoplasma.

3.1 Equipment

PCR instrument, clean benches, autoclaves, ice maker, nucleic acid protein analyzer, high-speed refrigerated centrifuge, small desktop centrifuge, ultra Cryogenic refrigerators, conventional refrigerator, Vortex, pipettes, electrophoresis, gel imaging system.

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3.2 Reagents

Glycerol, 75% ethanol,, acetic acid, chloroform, Kano fixative (ethanol. acetic acid. chloroform = 6.1.3), Insect preservation solution (75% ethanol, 1% glycerol), the PCR buffer, MgCl2, dNTPs, TaqDNA polymerase, the primers, the TAE electrophoresis buffer Fluid, DNA molecular weight standards, SYBRGreenI like.

4.1 Pretreatment insects

Insect starvation 12h, to prevent contamination of food within the insect gut DNA.

4.2 Phytoplasma insect genomic DNA extraction

Extraction of phytoplasma insect genomic DNA (see Appendix B).

4.3 Nested PCR and gel electrophoresis

Two pairs of phytoplasma universal primers amplified by nested PCR amplification and gel electrophoresis (see Appendix C).

4.4 Virtual RFLP fingerprint detection

See Appendix D.

5 Results found

5.1 4.3 test, if after a second round of PCR with universal primers R16F2n/R16R2, PCR amplification products by gel electrophoresis detection

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