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

GB/T 29393-2012

**Quarantine inspection and identification of Candidatus
Liberibacter asiaticus**

柑桔黄龙病菌的检疫检测与鉴定

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Foreword

This standard was drafted in accordance with GB/T 1.1-2009 given rules. This standard by the National Standardization Technical Committee of Plant Quarantine (SAC/TC271) and focal points. This standard was drafted. National Agricultural Technology Extension Service Center, Plant Protection and Quarantine Bureau of Zhejiang Province. The main drafters of this standard. WU Li-feng, Yan iron, Lin Yunbiao, item Yu, Zhu Jing whole, Julie. Quarantine detection and identification of bacteria huanglongbing

1 Scope

China's national method for the quarantine detection and identification of *Candidatus Liberibacter asiaticus*, the Asian species of the bacterium that causes citrus huanglongbing. It specifies the detection and identification methods for the organism and applies to its detection and identification in plant quarantine. Huanglongbing is the most destructive disease of citrus in the world. The bacterium lives in the phloem of the tree, is transmitted by the Asian citrus psyllid *Diaphorina citri*, and cannot be cured: an infected tree declines over a few years, produces small lopsided bitter fruit that will not colour, and dies. There is no resistant commercial variety and no treatment, so control is the removal of infected trees and the suppression of the vector - which means finding infected trees before they are obviously symptomatic, in an orchard of thousands. That is the detection problem. The bacterium has never been cultured, so it cannot be isolated and grown like an ordinary plant pathogen; it is distributed unevenly through the tree, so a sample from the wrong branch of an infected tree is negative; and the field symptoms, a blotchy mottle of the leaves that is not symmetrical about the midrib, resemble nutrient deficiency closely enough that they are constantly confused. So the standard combines the approaches available: examination of the field symptoms, transmission by grafting or by the psyllid vector to an indicator plant with observation of the resulting symptoms, and the laboratory identification of the organism. For a country that grows citrus on the scale China does, and that moves

planting material between provinces, the method is the practical instrument of containment. Issued on 31 December 2012 and in force since 1 March 2013.

This standard specifies the Asian citrus Huanglong bacteria species *Candidatus liberobacter asiaticum* jagoueix et al. Detection and Identification Setting Method. This standard applies to plant quarantine process for the detection and identification of bacteria huanglongbing Asian species.

2 Basic information of germs

Chinese name. citrus Huanglong germs. Name. *Candidatus liberobacter asiaticum* jagoueix et al. Disease English. Citrus Huanglongbing HLB. The case of thin-walled bacteria door Gracilicutes, phloem spp Liberobacter.

3 PRINCIPLE OF THE METHOD

According citrus plants Huanglong fungal infection onset symptoms typical field test; by grafting or by citrus psyllids (*Diaphorina citri*) after the transfer of disease, according to the onset of symptoms woody plant indicator identification; polymerase chain reaction (Polymerase Chain Reaction, PCR) detection Measurement Technology for huanglongbing bacteria in vitro amplification of DNA obtained rapid and effective identification results.

4 Instruments and appliances

UV transilluminator or gel imaging systems, centrifuges, nucleic acid electrophoresis, pH meter, pipettes. 10µL, 20µL, 100µL, 1000µL, PCR instrument, water bath, ultra-pure water systems, electronic scales.

5 Reagents and materials

TAE buffer, tris (hydroxymethyl) aminomethane - HCl (Tris-HCl), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), sodium acetate (NaAc), Taq enzyme, 10 x PCR buffer, 25mmol/L magnesium ion (Mg²⁺), 20mmol/L Deoxynucleotide triphosphate Phosphoric acid (dNTP), 5µL DNA fluorescent dye (GV), agarose, bromophenol blue, potassium permanganate, trichloromethane (chloroform), iso-amyl alcohol, isopropyl alcohol, Ethanol.

6 Symptom Recognition

The main identification is based on Huanglongbing field event of the "type blade mottled yellow" and "yellow shoot." Vane type mottled yellow to green blade that is After the main, lateral veins near the leaf base and start yellow, yellow green and yellow partial extension forming the uneven distribution of mottled, then can the whole leaf yellow Of. Yellow tip that is in the early stages, a small part of the shoot green canopy foliage yellow sheet. Some

results of citrus fruit from the tree was "red fruit nose." in Huanglong citrus seedling disease wards meet the above typical symptoms of diseased trees can be directly identified as citrus greening disease. Difficult to conclude suspected diseased seedlings, diseased trees, the choice of one or several detection methods for identification depending on the circumstances.

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