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Rules for Cotton Quarantine (Draft)

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Report Highlights:
This is an UNOFFICIAL translation of the People's Republic of China National Standard on The Rules for Cotton Quarantine (Preliminary Draft for Approval). The General Administration of Quality Supervision, Inspection and Quarantine of China (AQSIQ) notified this standard to the World Trade Organization on August 17, 2006 (G/SPS/N/CHN/98). According to AQSIQ, the purpose of this standard is to protect plants, animal and humans from diseases and pest damage. Any impact of this new standard on cotton trade must be further analyzed.

Includes PSD Changes: No
Includes Trade Matrix: No
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Beijing [CH1]
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Summary

The General Administration of Quality Supervision, Inspection and Quarantine of China (AQSIQ) notified this standard (Preliminary Draft for Approval) to the World Trade Organization on August 17, 2006 (G/SPS/N/CHN/98). The proposed date of adoption is ninety days after circulation by the WTO Secretariat, and the proposed date of entry into force is six months after adoption.

According to AQSIQ, the purpose of this standard is to protect plant, animal and human health from diseases and pest damage. Industry sources report that AQSIQ now conducts quarantine procedures for cotton entering China, and occasionally they encounter quarantine problems that lead to measures and costs incurred by the exporters. Although it is difficult for FAS Beijing to assess the trade impact of this new regulation at this moment, some industry insiders opined that it is unlikely to be a trade barrier as China currently China maintains the TRQ regime and a variable tariff system to regulate imports. However, further analysis by quarantine and SPS experts is warranted given the significant value of cotton flowing into China each year.

BEGIN TRANSLATION

Rules for Cotton Standard (Preliminary Draft for Approval)

Table of Contents

Preface

This document is a compulsive standard and the appendix as a recommendatory one. Attachment A is a documentary appendix. This Standard is put forward by the General Administration of Quality Supervision, Inspection and Quarantine. The Xinjiang Entry-Exit Inspection and Quarantine Bureau and the Xinjiang Institute of Agricultural Science draft this standard.

The main drafters are Xue Guanghua, Fan Weigong, Miao Weiguo, Zhang Xianglin, Mo Guihua, Liu Hongjun and Li-Bin.

The Rule for Cotton Quarantine

1 Scope

This standard stipulates the rules and procedures for cotton quarantine. This standard applies to quarantine of trade or non-trade cotton (excluding the seed cotton and absorbent cotton).

2 Normative Citation

The clauses in the following document are taken as clauses of this standard through citation in this standard. The revised editions and amendment lists of every dated citation (excluding the corrigendum) are not applicable to this standard. However, the concerned parties of this standard are encouraged to determine whether the latest versions of these documents are applicable. For every citation not dated, its latest edition is applicable to this standard.

GB/T18087 Plant Quarantine and Inspection Method for Trogoderma Granarium
GB7411 Plant Quarantine Rules for Place of Origin of Stock (Superior) Cotton Seed
SN/T1264 Quarantine and Inspection Method for Mexican Cotton Boll Weevil
SN/T1144 Quarantine and Inspection Method for Herba Orobanches
SN/T1385 Quarantine and Inspection Method for Dodder Seed
SN/T1356 Quarantine and Inspection Method for Cotton Root Rot

3 Technical Terms and Definition

The following technical terms and definitions are applicable to this standard.
3.1 Entry Quarantine
Quarantine for cotton imported from foreign countries or other regions of the country.

3.2 Exit Quarantine
Quarantine for cotton exported to foreign countries or other regions of the country.

3.3 Pest
Harmful germ, pest, weed etc, in the process of production, transportation or storage.

3.4 Quarantine Pest
Harmful pest which has potential economic importance towards the threatened region, yet hasn't occurred in that region, or occurred but not widely distributed and under official control.

3.5 Lot
Imported or exported cotton from and to the same nation or region, loaded by the same carrier, of the same consignee or consignor, same species name, and same merchandise specification.

4 Quarantine Requirements

4.1 Domestic Quarantine Requirements
Quarantine objects in the growth and storage period as stipulated in the List of Agricultural Phytosanitary Object and Plants, Plant Products Subject to Quarantine Inspection; the Implementation Measures of Plant Quarantine as Issued by each province, autonomous region and municipalities directly under the central government; the List of Agricultural Phytosanitary Object (and supplementary list).

4.2 Import Quarantine Requirements
Harmful living organisms related to the growth and storage period of cotton, as stated in Chinese laws concerning lists of hazardous or potential hazardous diseases, pests, and weeds; harmful pests related to cotton as stipulated in plant quarantine bilateral agreement, protocols and memos, between China and relevant countries and regions; quarantine clauses and requirements in trade contracts and letters of credit.

4.3 Export Quarantine Requirements
Harmful living organisms as stipulated in plant quarantine bilateral agreement, protocol and memos, concerning about harmful pest related to cotton, signed by and between China and relevant countries and regions. Quarantine clauses and requirements in trade contract, and letter of credit.

5 Quarantine Preparation

5.1 On-Site Quarantine Tools
Sample bag, knife, pincers, white porcelain dish, Chinese brush-pen, forceps, finger-shape tube, collect bag, magnifier, flashlight, pencil, sample label, etc. and relevant record form.

5.2 Laboratory Quarantine Facilities
See appendix A.

6 Import Quarantine

6.1 On-Site Quarantine
6.1.1 Goods and Certificate Check-Up
Check if the certificates comply with the goods and find out how the goods are carried and stacked up, if the conveyance tools and the packing containers have carried agricultural or livestock products, or if cotton has been fumigated.

6.1.2 Warehouse Check-up
Check around the warehouse, the corners, ground, overlay cloth and the cushions to identify if there are live harmful organisms and traces of infection.

6.1.3 Check-up of Conveyance and Cushion
While boarding cargo ships, trucks, airplanes and containers for quarantine inspection, examine the apertures, girders, corners and cushions of the cabins, trucks, compartments and boxes to look for animal and plant leftovers or traces of live harmful organisms.

6.1.4 Package Check-up
Open the cabins or cases, check the upper layer and outside of the stacking and the cotton casing to determine whether there exist any live harmful organisms or traces of infection, soil, weeds, mildews, animal corpses, etc.

6.1.5 Cargo Check-up
6.1.5.1 Cut and open the cloth bale and check the inside and outside of the wrap and surface layer of the cotton to look for live harmful organisms and traces of infection, such as pest feces and sloughs etc. Check if cotton seeds, cottonseed hulls, weed seeds and other plant remnants are mixed. Collect the harmful living organisms, weed seeds, cotton seeds and other plant remnants, etc., and put them into the finger-shaped tube or collection bag for laboratory inspection and quarantine.

6.1.5.2 Make random inspection and sampling as per the requirement of 6.2. If no harmful organisms are found in the surface layer of cotton, continue to conduct quarantine and inspection of the cotton in the middle and bottom layer of the load or inner layers of cotton in the container.

6.1.6 On-site Quarantine Record

6.2 Random Inspection and Sampling

6.2.1 Random Inspection Method
6.2.1.1 Inspect 3%-10% of the number of items in each lot.
6.2.1.2 Combine random inspection and selection of position where harmful living organisms tend to hide. Emphasis should be placed on cotton with seeds (hulls), weed seeds and plant remnants when opening the package for random inspection.
6.2.1.3 When conducting inspection of a stack, take representative samples from the top, middle, bottom layers of the stack based on the diagonal or chessboard lines or random sampling method.
6.2.1.4 When inspecting cotton loaded in a vessel, aircraft, railcar/truck and container; take representative samples in accordance with 6.2.1.3 respectively.

6.2.2 Sampling
Based on the quarantine lot as a unit, take samples from the randomly inspected cotton.
--for every lot with less than 50 packs, take one sample; 50 to 100 packs, take two samples; 101 to 200 packs, take three samples.
--container transport by land or air, add one more sample for every incremental 100 packs.
--marine transport (the whole ship in bulk), add one more sample for every incremental 500 packs.
Each original sample weighs 2.0 kg to 2.5 kg.

7 Export Quarantine

7.1 Field Quarantine Investigation
7.1.1 In the growing period of cotton, investigate the outbreak and the prevention of major diseases, pests and weeds. (Referring to GB7411)
7.1.2 Field Quarantine Record

7.2 Processing Quarantine
7.2.1 Examine cotton processing facilities and processing procedures.
7.2.2 The facility and its surrounding areas should be free from other piled agricultural and livestock products; the facility should be free from sundries likely to cause infestation; the processed cotton, seed cotton and semi-processed cotton should be stored separately; unpacked processed cotton shouldn't contain live harmful organisms, soil, cotton seeds(hull)and plant remnants; dissect the cotton seed and check if there is any pest in it.
7.2.3 Send samples together with harmful organisms for laboratory quarantine if necessary.
7.2.4 Quarantine record in processing. The contents include: origin of cotton, location, environmental and sanitary conditions of processing facility, processing capacity, the percentage of cotton seeds (hull), plant remnants in the cotton and presence of harmful organisms.

7.3 Warehouse Quarantine
7.3.1 The warehouse area should be free from stacked other agricultural products, livestock products and sundries likely to cause infestation. The wall, the foot and corner of wall, and window sill, etc, should be free from traces of harmful pest; the exterior of cotton package and cushion should be free from live harmful pest and soil.
7.3.2 Make random inspection of cotton bales when necessary, and check the interior and exterior of the package and surface layer of the cotton for live harmful organisms and its traces, cotton seeds (hull), plant remnants; dissect the cotton seed to check if it contains harmful pest.
7.3.3 Take samples for Laboratory Quarantine.
7.3.4 Warehouse Quarantine Record.

7.4 On-Site Quarantine

7.4.1 Before an owner submits an application for quarantine inspection, export cotton should be piled collectively. After accepting the application, the quarantine officer shall verify the certificates and quarantine records as specified in 7.1 or 7.2 or 7.3.
7.4.2 Conduct quarantine inspection as specified in 6.1.
7.4.3 Perform random inspection and sampling as specified in 6.2.
7.4.4 Take samples for laboratory quarantine and inspection in the case of any problems.
7.4.5 On-site Quarantine Record.

7.5 Export or Exit Quarantine
7.5.1 Check the packing of cotton to determine if it's infested with harmful living organisms. Pay attention to the exterior of packing and aperture for any hiding harmful pest.
7.5.2 If any harmful living organism or trace is found, open up the package and conduct quarantine and inspection as stipulated in 6.1.
7.5.3 Conduct random inspection and sampling as stipulated in 6.2.
7.5.4 Quarantine Record

8 Laboratory Quarantine and Inspection
See the appendix A: Cotton Laboratory Quarantine and Inspection.

9 Quarantine Result Assessment and Certification

9.1 Result Assessment
9.1.1 Check the results of on-site quarantine, processing quarantine and laboratory quarantine in accordance with relevant quarantine requirements. If the quarantine results are satisfactory, judge as acceptable, otherwise, unacceptable.
9.1.2 As for cotton not compliant with relevant quarantine requirements, if effective treatment method is available, allow entry or exit subject to effective disinfection treatment. In the case of lack of effective treatment, the cotton should be returned to the owner, and entry or exit is not allowed.

9.2 Certification
9.2.1 Export Quarantine
9.2.1.1 Plant Quarantine Certificate shall be provided for domestic cotton transshipment (inter provinces).
9.2.1.2 Plant Quarantine Certificate shall be provided for export of cotton (format: C5-1).
9.2.1.3 If the importing country or region requires Fumigation Certificate, Plant Quarantine Certificate (format C5 - 1) and Fumigation/Disinfection Certificate (format C7 - 1) shall be provided after eligible fumigation and disinfection.

9.2.2 Import Quarantine
9.2.2.1 Inbound Goods Quarantine Certificate (format 5-1) shall be provided for import of cotton.
9.2.2.2 Plant Quarantine Certificate shall be provided for domestic cotton transshipment (inter provinces).

Appendix A (for documentation)
Laboratory Quarantine and Assessment for Cotton

A.1 Requirement of Laboratory

Equipment for fungi, germ, virus separation and incubation (conservatory box, incubation box), equipment for insect breeding, identification (microscope), equipment for weed cultivation and identification and relevant data etc.

A.2 Preparation of Cotton Sample

Using the method of quartile, prepare two units of cotton samples collected from on-site quarantine, one unit for laboratory use, the other for backup check.

A.3 Inspection

Carefully examine whether there exists pest, weed seed and cotton seed (hull). If yes, send them to relevant laboratory for quarantine and assessment, together with the pest, cotton seed(hull), plant remnant and weed seed collected on-site.

A.3.1 Insect Quarantine and Assessment
A.3.1.1 GB/T18087 (Quarantine and Assessment Method for Trogoderma Granarium)
A.3.1.2 SN/T1264 (Quarantine and Assessment Method for Mexican Cotton Boll Weevil)
A.3.1.3 Other Insects Quarantine and Assessment
A.3.2 Weed Quarantine and Assessment
A.3.2.1 SN/T1144 Herbal Orobanches's Quarantine and Assessment Method
A.3.2.2 SN/T1385 Quarantine and Assessment Method for Dodder Seed
A.3.2.3 Other weeds Quarantine and Assessment
A.3.3 Plant Pathogeny Quarantine and Assessment

A.3.3.1 Fungi Quarantine and Assessment
A.3.3. 1.1 Culture Media for Inspection of Main Cotton Pathogenic Fungi
Cotton fusarium wilt: use ZhiXuan No.1 or PDA culture media.
Cotton verticillium wilt: use ZhiXuan No.1 or D culture media.
Cotton Root Rot: use MiangXuan No.1 or D culture media.
A.3.3.1.2 Preparation of culture media
A.3.3.1.2 .1 TBCEN formula

	1.1L
1st	
Autoclave	
Agar	12g
H2O	1L
2nd	
cooled to 50°C, then add in order	
CaCO3	1.1 g
Conc. HCl	1.1ml
Penicillium G	0.066g
Streptomycin sulfate	0.55g
3rd	
Add sterile water approx.vol.:5ml	
Chlortetracycline HCl*	0.055g
*Add 5N NaOH until it dissolves approx. 2 drops	
Then add: Nystatin 250,000units(5880/mg)	0.061g
Votex and add to media	
4th	
Terrazole	1.5g
5th	
Fresh juice made from peeled carrots	100ml

A.3.3.1.2 .2 ZhiXuan No.1 formula

Take the peptone as the nitrogen source, the cane sugar as the carbon source, and then add Potassium Phosphate Monobasic, magnesium sulfates and chlorination potassium, etc. Use Pentachloronitrobenzene and Potassium metabisulfite to restrain the growth of other epiphytes, rather than fusarium oxysporumthe. Use Streptomycin to restrain germs, and Methyl cellulose to expedite the growth of large conidiophore.

Composition:

Methyl cellulose (CMC)	1g	pentachloronitrobenzene (PCNB)	0.1g
Peptone	5g	Cane sugar	20g
Potassium Phosphate Monobasic	1g	agar	20g
Magnesium sulfate	0.5g	Distilled water	1000ml
Ammonium nitrate	0.3g	Streptomycin	0.1g
Potassium chloride	0.6g	Nysfungin	100,000 units
Potassium metabisulfite	0.2g		

Annex 1: add Methyl cellulose (CMC),Streptomycin, Nysfungin after disinfection. Nysfungin may not be added, but need to be added when the temperature is high and there are a lot of mildews.

A.3.3.1.2 .3 MianXuan No.1 formula

Composition:

Sodium nitrate	2g	Magnesium sulfate	0.5g
Potassium Phosphate Monobasic	1g	Fe2(SO4)3.XH2O	0.01g
Potassium chloride	0.5g	Cane sugar	5g
Agar	15g	Distilled water	1000ml
Chloromycetin	300mg	JingGang Mycin	2.5ppm
Pentachloronitrobenzene (PCNB)			350ppm
Phthalimide			0.5ppm

Annex 1: add JingGang Mycin, pentachloronitrobenzene(PCNB), phthalimide after disinfection.

Annex 2: add 2.5 g soil sample into 200 mls water solution dissolved with 1% Sodium polyphosphate and 0.01% Tergitols-NPXs, and then stir for 15min at 8000 rpm.

A.3.3.1.2 .4 D Culture Media Formula

Composition:

Soil immersion liquid	25ml	Potassium hydrogen phosphate anhydrous	4g
Potassium Phosphate Monobasic	1.5g	Sodium urate	0.2 g
Pectin (GA)	1.0 g	Sorbitol	1.0g
Dox salt	2ml	Tergitol NP-10	1.0ml
Pentachloronitrobenzene (PCNB)	0.1g	Agar	17g
Distilled water	1000ml		

pH=6.4~6.7

Annex 1: Preparation of soil immersion liquid: place 1 kg fertile garden soil into a 2 L triangular flask, and add 1000 mls distilled water, immerse in the boiled water for 1h. After several filtering and settlement, place 1000 ml solution at 4 °C for standby use.

Annex 2: Dox salt: (containing Potassium Phosphate Monobasic, 2 g, potassium chloride, 2 g Magnesium sulfate, 1 g, Fe2 (SO4) 3.XH2O, 0.02 g; Sodium nitrate 4 g, distilled water 20 ml)

Annex 3: add pentachloronitrobenzene (PCNB) before culture media disinfection, place under 121 °C for 30 min disinfection. After the culture media is melted and the temperature declines to 45 °C, add 50 mls bacteriophage liquid to each 1000 mls culture media liquid

(containing chloromycetin 0.05 g, Streptomycin 0.05 g, benzylpenicillin 0.05 g). After complete mixing, pour 15 ml culture media into each utensil. .

A.3.3.1.3 Separation Quarantine Assessment
A.3.3.1.3.1 Separation of Cotton Seed (hull) Pathogenic Fungus
Washing and microscope inspection: put cottonseed (hull) into the triangular flask, add sterile water and vibrate for 5 min to 10 min. Put the turbid water in the centrifugal tube at 3000 rpm for 5 min, remove the pure liquid, and take sediments for check under the microscope.
Cultivation inspection: take some cottonseeds (hull), put into a yarn net bag, wash 12 h to 24 h with tap water, and disinfect in 0.1% Hg liquid for 2 min to 3 min. Use sterile water to clean up three times and clip the cotton seed (hull) to culture medium, each of which contains 5 to 6 grains. Cultivate in 25 °C constant temperature box for 3 d--4 d. Transfer the colonies to PSA bevel tube and cultivate at 25 °C for 3d--4 d, and check under microscope to determine its category according to the fungus appearance and cultivation characteristic.

A.3.3.1.3.2 Separation and Inspection of Pathogenic Fungus on the Cotton Fiber and Remnant of Plant Trunk
Washing and microscope inspection: divide 1 g cotton fiber sample into three units, put respectively in a triangular flask that contains 100 ml sterile water. After thorough saturation, place the triangular flask on a constant temperature shaker and stir at 120 rpm for 24 h, then enable 5 to 10 min centrifugation at 3000 rpm. Remove the pure liquid and take the sediments for microscope check;
Cultivation inspection: cut 1g cotton fiber sample into 1 mm long, and place respectively in 6 sterile cultivation utensils of 9 cm in diameter. Gently pour in 8 ml to 10 ml optional culture medium at 42 °C to 45 °C, make the cotton fiber evenly distributed; after the culture medium is solidified, pour in 15 ml to 20 ml of the same culture medium at 42 °C to 45 °C, and bury the cotton fiber and plant trunk remnant into the culture medium; cultivate them in constant temperature box at 25 °C for 7 d. Observe and check with microscope about its category according to its appearance and cultivation characteristics.

A.3.3.2 Bacteria Inspection
A.3.3.2.1 Place 50 grams of cottonseeds into a triangular flask with 100 ml sterile normal saline (0.85% NaCls) at 5°C. Slowly shake the triangular flask to immerse all cottonseeds. Place them in darkness at 5 °C to cultivate for 18 hours.
A.3.3.2.2 Put 0.05 ml disinfected Tween (R) 20 and vibrate for one minute.
A.3.3.2.3 Filter the suspended liquid with double layer gauze, and put into a sterile centrifuge tube.
A.3.3.2.4 Centrifuge 8000 g for 15 minutes, remove the top clear liquid, and suspend the sediments with 10 ml normal saline.
A.3.3.2.5 Dilute the suspended sediment liquid to varying degrees at 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Take 0.1 ml at each dilution degree and coat onto MOPS optional culture medium utensil, and repeat three times. Then, put the utensil in constant temperature box at 28 °C for three days.
A.3.3.2.6 The cotton Pseudornonas syrmgae Pv.lachymans is formed on the MOPS optional culture medium. Before performing pathogenic measurement, purify suspicious bacterium throngs individually.

A.3.3.2.7 Pathogenic Test.
Put all suspicious separated substances of Pseudornonas syrmgae Pv·lachymans onto young cotton sprouts at 2-5 slices of leafs to carry out pathogenic test. Operating method is as follows:

A.3.3.2.7.1 After 36 hours of cultivation, prepare the bacteria separated substances into 108cfu/mL bacteria suspending liquid and spray onto the leaves of young cotton sprout until drops of water drip from the leaves. Each separated substance inoculates at least five young cotton sprouts. Place the inoculated young sprouts in highly wet environment set at 25-30 ℃ to grow for 8-15 days and then observe the development of disease. The young sprouts, being infected by Pseudornonas syrmgae Pv·lachymans, show circular or oval spots of oily dark green color on the cotyledon, later the disease spots expand gradually, and the color changes into brown or deep brown; disease spots on the true leaves expand and take the shape of polygons because of being subject to leaf vein, generally 1-4 mm in size, or connected in patches in the case of severity.

A.3.3.2.7.2 Separate germina again from infected plant, and quickly carry on inspection to make certain whether it is Xanthomonas campestris pv. malvacearum(Smith) Dye.

A.3.3.2. 8 MOPS optional culture media formula:

50% glycerol	20 ml	30% K2HPO4	1 ml
Agar	15 g	H2O	880 ml

After high-pressure sterilization, cool down to 50 ℃, add the following samples:
Add 100 ml disinfected 10 times MOPS 10 X reservation liquid.
Add 5 ml disinfected seven amino acids 200 X reservation liquid.
Add just the right amount of antibiotics (such as: 25 The mg/ml kanamycin Kan35 1.4 ml).

Annex 1: MOPS 10 X reservation liquid

MOPS (MW 209.3)..... .....83.7 g	Tricine (MW 179.2)..... ..... 7.2 g
FeSO4.7 H2O (MW 278.0).. ..... 0.03 g	NH4Cl (MW 53.5)..... ..... 5.0 g
K2SO4 (MW 174.3)..... 0.5 g	MgCl2.6 H2O (MW 203.3)..... 1.0 g
KBr (MW 119.0)..... 1.0 g	CaCl2.2 H2O (MW 147.0)..... 1 ml of 1 mM

Microelement (see annex 2).....10 mls(the 105 X reservation liquid)
Dissolve in the 900 ml distilled water, adjust pH to 7.4 with the NaOH, then fix into 1000 ml solution. Filtrate and disinfect, store at the temperature of 4 ℃.

Annex 2: 105 X microelement reservation liquid
(NH4) 6Mo7O24.4 H2O (MW 1235.9).....0.03 Ms(ammonium molybdate)

CoCl2.6 H2O (MW 237.9)..... 0.3 M	CuSO4.5 H2O (MW 249.7)..... 0.1 M
ZnSO4.7 H2O (MW 287.9)..... 0.1 M	MnCl2.4 H2O (MW 197.9)..... 0.8 M

Prepare above-mentioned microelements into 105 X reservation liquid, filter and disinfect, store at the temperature of 20 ℃.

Annex 3: 200 X seven amino acids reservation liquid

alanine (MW 89.0)..... 0.84 g	arginine.HCl (MW 210.7)..... 2.53 g
asparagine.H2O (MW 150.1)..... 0.85 g	histidine.HCl (MW 209.6)..... 0.42 g
isoleucine (MW 131.2)..... 0.79 g	phenylalanine (MW 165.2)..... 0.99 g
threonine (MW 119.1)..... 0.71 g	

Dissolve in 90 ml distilled water, then fix to 100 ml solution. filtrate and disinfect, store at 20 ℃.

A.3.3.3 Virus Inspection
A.3.3.3.1 Nucleic acid distillation
A.3.3.3.1.1 Take 5 g cotton seed, place in mortar and add liquid nitrogen to grind into powder.
A.3.3.3.1.2 Take 100 mg sample and put into 2 ml centrifugal tube, add 1000μL CTAB extraction liquid and 2μL RNase enzyme solution, mix fully and incubate at 65 ℃ for 30 min, and vibrate occasionally.

A.3.3.3.1.3 Centrifugate at 12000 rpm for 5 minutes under room temperature, transfer the clear liquid to another 2 ml clean centrifugal tube and add 24:1 same volume of chloroform/ 3-Methyl-1-butanol, gently reverse several times and hold still for 5 min at room temperature.
A.3.3.3.1.4 Centrifugate at 12000 rpm for 10 minutes under room temperature, transfer the clear liquid to another 1.5 ml clean centrifugal tube and add same volume of CTAB, gently reverse several times and hold still for 1h at room temperature.
A.3.3.3.1.5 Centrifugate at 12000 rpm for 5 minutes under room temperature, remove the clear liquid and add 400 μ L 1 M NaCl dissolution sediment, incubate at 56 ℃ for 15 min.
A.3.3.3.1.6 Add 800 μ Ls -20 ℃ water-free Ethyl Alcohol, reverse to mix at -70 ℃ for 30 min or -20 ℃ for 1 h.
A.3.3.3.1.7 Centrifugate at 12000 rpm for 10 minutes under room temperature, remove the clear liquid, wash and deposit with 70% Ethyl Alcohol for 2-3 times to dry DNA;
A.3.3.3.1.8 Add 50 μ L TE buffer liquid to dissolve the sediment and store in refrigerator at 4 ℃ for backup use.
A.3.3.3.2 PCR Examination
A.3.3.3.2.1 Introductory substance sequence: the followings can be selected for PCR expansion.
CL-CR positive: 5'-CCC TGA ATG TTY GGA TGG AA-3'
CL-CR negative: 5'-CGG GCG TAG AAA TGA CGA T-3'
A.3.3.3.2.2 PCR reaction system

10 × PCR Buffers	5 μ Ls
25mM MgCl2	3 μ Ls
2.5mM dNTP	2 μ Ls
The 20 pmol/μ L lead-up positive	1 μ L
The 20 pmol/μ L leads-up negative	1 μ L
1 U/μ L Taq enzyme	One μ L
DNA template	5 μ Ls
ddH2O	32 μ Ls

A.3.3.3.3 PCR reaction condition
94 ℃/5 min; 94 ℃/30 s, 55 ℃/40 s, 72 ℃/60 s, 40 cycles; 72 ℃/3 min, stored at 4 ℃.
A.3.3.3.3 Gel electrophoresis inspection of PCR resultant
Prepare 1.5% agar sugar gel, mix well the buffer liquid and PCR resultant on a pro-rated basis. And, add it into the sample bore, use 100bp DNA Marker as the molecule mark and conduct the electrophoresis analysis. Under the ultraviolet light of Gel Imaging instrument, observe if differential DNA electrophoresis belt is expanded. Shoot film and make record.

A.4 Indoor Quarantine Record

Fill in the indoor quarantine and inspection record form. Fill in report of inspection result about intercepted and captured pathogenic fungus, pest and weed, etc. Harmful living organisms or firstly intercepted harmful living organisms after quarantine shall be subjected to experts' appraisal and review.

A.5 Conservation of Intercepted and Captured Substances

Make specimen of identified pathogenic fungus, pest and weed, etc, and then store them properly.

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