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**Detection of pathogen in food - PCR-DHPLC method
(English Translation)**

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Foreword

This Standard is prepared according to the rules specified in GB/T 1.1-2009.

This Standard is under the jurisdiction of Certification and Accreditation Administration of the People's Republic of China.

The responsible drafting organizations are Liaoning Entry-Exit Inspection and Quarantine Bureau of P.R. China, Fujian Entry-Exit Inspection and Quarantine Bureau of P.R. China, Jiangxi Entry-Exit Inspection and Quarantine Bureau of P.R. China, Jilin Entry-Exit Inspection and Quarantine Bureau of P.R. China and Beijing Yingjiusi Technology

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Detection of pathogen in food -

PCR-DHPLC method

1 Scope

This Standard specifies the detection method of salmonella and other 30 kinds of pathogen in food-PCR-DHPLC method.

This Standard applies to the rapid detection of salmonella and other 30 kinds of pathogen in food.

Note: 30 kinds of common pathogen include. Salmonella, shigella, staphylococcus aureus, yersinia enterocolitica, listeria monocytogenes, campylobacter jejuni, bending thermophilic bacteria, enterohemorrhagic Escherichia coli O157.H7, enterotoxigenic escherichia coli, intestinal pathogenic

escherichia coli, intestinal invasive escherichia coli, enterobacter sakazakii, vibrio parahaemolyticus,

vibrio cholerae, vibrio vulnificus, vibrio alginolyticus, vibrio mimicus, vibrio fluvialis, vibrio metschnikovi,

aeromonas hydrophila, clostridium botulinum, clostridium perfringens, bacillus cereus, hemolytic

streptococcus, brucella, lactic acid bacteria, pseudomonas aeruginosa, klebsiella pneumoniae, bacillus

proteus vulgaris, and proteus mirabilis.

2 Normative References

The articles contained in the following documents have become this Standard when they are quoted herein. For the dated documents so quoted, all the modifications (including all corresponding corrections) or revisions made thereafter shall be applicable to this Standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

GB 19489 Laboratories - General requirements for biosafety

GB/T 27403 Criterion on quality control of laboratories - Molecular biological testing of food

10.1 Sample preparation, enrichment culture and separation

Conduct the enrichment and separation culture according to the relevant national standards, industry standards or international authoritative standard methods in Annex B.

10.2.1 Preparation of enrichment broth template DNA

TAKE 1.5mL corresponding pathogen enrichment broth cultured in 10.1 (if the secondary culture is required, take a secondary enrichment broth), and PLACE into 1.5mL sterile centrifuge tube, 13,000g-CENTRIFUGATE for 1min; ABANDON the supernatant and TAKE the precipitation, ADD 567μL TE solution (pH8.0), SUSPEND, ADD 30μL 10% SDS and 3μL proteinase K (20mg/mL), MIX evenly, 37°C WARM-BATH for 1h; ADD 100μL sodium chloride (5mol/L), MIX evenly, ADD 80μL CTAB/NaCl solution (10% CTAB and 0.7mol/L NaCl), MIX evenly, 65°C WARM-BATH for 10min; ADD an equal volume of chloroform/isoamyl alcohol (with volume ratio of 24.1), MIX evenly, 13,000g-CENTRIFUGATE for 10min; TAKE the supernatant, ADD an equal volume of phenol/trichloromethane/isoamylol (with the volume ratio of 25.24.1), MIX evenly, 13,000g-CENTRIFUGATE for 10min; TAKE the supernatant, ADD 0.6 times the volume of isopropanol, MIX evenly, 13,000g-CENTRIFUGATE for 10min; TAKE the precipitation, WASH with 70% ethyl alcohol for two times, DRY it, Add 100μL TE solution (pH8.0) to dissolve -- this is the DNA solution. If it can not be detected immediately, STORE at -20°C for spare use. PREPARE enrichment broth template DNA of positive control bacterial strain and a negative control bacterial strain in the same way. May also use commercial DNA extraction kit and PREPARE the template DNA according to the instructions.

10.2.2 Preparation of suspicious bacterial colony template DNA

PICK suspicious bacterial colony or thallus obtained from separation in 10.1, or 1.5mL of

its passage nutrient solution, PREPARE DNA template according to steps in 10.2.1 for detection. May also use commercial DNA extraction kit and PREPARE the template DNA according to the instructions.

10.3 PCR amplification

10.4.1, set up detection procedure and run.

10.5 The contrast settings of quality control

Positive contrast and the negative contrast shall be set up in process of detection respectively. The positive contrast is standard strain of the target pathogen and the negative contrast is standard strain of non-target pathogen.

11.1.2 Positive contrast. The typical absorption peak of PCR product appears, and the

peak absorption value is greater than 3mV.

11.1.3 For those that may not meet the above contrast quality control standard, it is

deemed to be invalid.

11.2 Results determination and report

For example of salmonella, the example of DHPLC detection patterns refers to Annex C.

- a) If there is no amplification absorption peak in detected samples, the results can be determined as negative and directly report that the XXX pathogen can not be detected;
- b) If the typical absorption peak of PCR product appears in detected samples and the absorption peak is more than 3mV, the sample XXX pathogen can be determined as suspicious positive;
- c) If the typical absorption peak of PCR product occurs in detected samples but the absorption peak is less than 3mV, then propose to adjust the amplification parameters of the PCR to redetect. If the absorption peak of the redo-results is still less than 3mV, the XXX pathogen is negative; otherwise the XXX pathogen can be determined as suspicious positive;