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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 3841-2014

Rapid detection of norovirus and astrovirus in shellfish for export. Reverse transcription-loop-mediated isothermal amplification method

出口贝类中诺如病毒和星状病毒的快速检测 反转录-环介导恒温核酸扩增（RT-LAMP）法

(English Translation)

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Foreword

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

Please note that some of the content of this document may involve patents. Release mechanism of the present document does not assume responsibility for the identification of these patents.

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

This standard was drafted. People's Republic of China Beijing Entry-Exit Inspection and Quarantine Bureau.

1 Scope

This standard specifies the norovirus in shellfish and astrovirus reverse transcription - loop-mediated isothermal nucleic acid amplification (RT-LAMP) quickly seized Measurement method.

This standard applies to shellfish Norovirus rapid detection of the virus and astrovirus.

2 Normative references

The following documents for the application of this document is essential. For dated references, only the dated version suitable for use herein Member. For undated references, the latest edition (including any amendments) applies to this document.

Laboratory use specifications and test methods GB/T 6682 Analysis GB 19489 General requirements for laboratory biosafety GB/T 27403 laboratory quality control of food molecular biology standardized testing SN/T 1635 shellfish Norwalk virus detection methods common RT-PCR method and real-time RT-PCR.

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SN/T 2519 shellfish stellate common method of virus detection and real-time PCR method PCR

3 Abbreviations

The following abbreviations apply to this document.

3.1
Betaine. Betaine.

3.2
Bst enzyme. BstDNApolymerase (largefragment), BstDNA polymerase (large fragment).

3.3
dNTP. deoxyribonucleosidetriphosphate, deoxynucleoside triphosphates.

3.4
LAMP. loop-mediatedisothermalamplification, loop-mediated isothermal amplification.

3.5
RNA. Ribonucleicacid, ribonucleic acid.

4 Technology Overview

Use GPTT Act (Glycine-PEG-Trizol-oligodT) viral RNA was extracted shellfish samples or nano-beads; independently selected from Take astrovirus capsid protein coding gene (ORF2) 5 'end, GI type and GII highly conserved type norovirus virus ORF1 and ORF2 joints Shou sequence design 5 ~ 6 RT-LAMP primers, eight separate areas 7 ~ specifically recognizes viral RNA on having a chain set in other action Bst enzyme, under AMV reverse transcriptase action at isothermal conditions (63 °C or 65 °C) insulation 60min, you can step to achieve the virus RNA amplification efficiency. Amplification reaction produces a large number of by-product (magnesium pyrophosphate) milky white precipitate formed, the reaction tube was added SYBR GreenI fluorescent dyes, fluorescent green anode reaction was rather negative reaction is a fluorescent orange.

5 Reagents and materials

Unless otherwise stated, all test reagents used were of analytical grade; experimental water meet the requirements of GB/T 6682 in one of the water.

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