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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 5325.3-2020

Quantitative detection of food-borne viruses in exported food. Digital PCR method - Part 3: Rotavirus

出口食品中食源性病毒定量检测 数字PCR法 第3部分：轮状病毒

(English Translation)

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Foreword

SN/T 5235-2020 "Digital PCR Method for the Quantitative Detection of Foodborne Viruses in Exported Foods" is divided into 7 parts.

Part 1: Norovirus;

Part 2: Hepatitis A virus;

Part 3: Rotavirus;

Part 4: Zaru Virus;

Part 5: Astrovirus;

Part 6: Coxsackie virus;

Part 7: Poliomyelitis virus.

This part is part 3 of SN/T 5235-2020.

This part is written according to the rules given in GB/T 1.1-2009.

1 Scope

This part specifies the digital PCR detection of rotavirus in foods such as shellfish, hard surface foods, raw vegetables, soft fruits, etc.

method.

This part is applicable to the quantitative detection of rotavirus in foods such as shellfish, hard surface foods, raw vegetables, and soft fruits.

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The detection limit achieved by this method is 1 800 copies/2 g (shellfish digestive glands); 1 800 copies/100 cm2 (hard surface food);

3 600 copies/25 g (raw vegetables and soft fruits).

The limit of quantification achieved by this method is 3 600 copies/2 g (shellfish digestive glands); 3 600 copies/100 cm2 (hard surface food);

7.200 copies/25 g (raw vegetables and soft fruits).

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analyze and test water specifications and test methods.

3 Terms, definitions and abbreviations

The following terms and definitions apply to this document.

Limit of determination (LOD) the method can detect the lowest rotavirus gene content in the sample.

Limit of quantification (LOQ) Under the condition that the relative standard deviation does not exceed 25%, the method can quantitatively detect the lowest rotavirus gene content in the sample.

Tiny reaction system Mix the template, primers/probes, Taq enzyme and its buffer thoroughly, and distribute them to oil packets of the same volume and physically separated from each other A small-volume fluorescent PCR reaction system formed in water droplets or other micropores and microchambers.

Process control

By adding exogenous quality control substances similar to the target virus, such as MS2 phage, the entire detection process can be monitored.

Process the calculation of the recovery rate of quality control substances to evaluate the effectiveness of the detection process and calculate the actual content of the virus in the sample.

External control RNA (EC RNA) Exogenous positive control RNA is used as a positive control for the digital PCR process.

Certified reference material Attached with a certificate issued by an authoritative

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organization, explaining the use procedure and obtaining one or more characteristics with relevant uncertainty and traceability Value of the standard substance.

4 Principle

Digital PCR (digital PCR) is a quantitative detection technology of gene copy number developed on the basis of fluorescent PCR. It is used for nucleic acid modeling.

Determination of the absolute copy number of the plate. By fully mixing the fluorescent PCR reaction system containing template, primer/probe, Taq enzyme and its buffer After homogenization, the amount is equally divided into a large number (more than 10,000) of micro-reaction systems isolated from each other, so that each template is independently and randomly allocated to Micro reaction system. After all micro-reaction systems undergo PCR amplification reactions under the same prescribed conditions at the same time, according to the set fluorescence threshold Value to judge the amplification result of each micro-reaction system. The digital PCR reaction is calculated according to the positive rate of the microreaction system and the Poisson distribution formula.

Should be the template concentration in the system.

5 Equipment and materials

5.1
Digital PCR system. including a micro-reaction system generator or other instruments with the same function, a micro-reaction system fluorescence detector or Other systems with the same functional instrument.

5.2
Nucleic acid quantifier. Nanodrop3000 or Modulus detector or other nucleic acid quantitative detector.

5.3
Refrigerated centrifuge.

5.4
Biological safety cabinet.

5.5
Low temperature refrigerator. -80 °C refrigerator, -20 °C refrigerator.

5.6
Balance. Sensitivity is 0.1 g.

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5.7
Homogenizer.

5.8
Vortex oscillator.

5.9
Autoclave.

5.10
Constant temperature incubator/box. temperature control accuracy ±1.0 °C.

5.11
Micropipette. 100 μL~1 000 μL, 20 μL~200 μL, 10 μL~100 μL, 0.5 μL~10 μL, 0.1 μL~2.5 μL.

5.12
The digital PCR micro-reaction system generates row tubes and membranes or chips.

5.13
Mesh filter bag. 400 mL.

5.14
Sterile cotton swabs.

5.15
Sterile scissors.

5.16
Sterile forceps.

5.17
Sterile petri dishes.

5.18
Glass container without RNase and DNase contamination.

5.19
RNase-free centrifuge tube, RNase-free pipette nozzle, RNase-free spoon, RNase-free PCR thin-walled tube.

6 Reagents

All experimental reagents are analytically pure and meet the requirements of GB/T 6682; unless otherwise specified, the experimental water is RNase-free ultra Pure water.

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