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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 3631-2013

Detection of Enterovirus 71 in shellfish for epxort. Real-time fluorescence RT-PCR

出口贝类中肠道病毒EV71的检验方法 实时荧光RT-PCR方法

(English Translation)

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Foreword

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

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

This standard was drafted. People's Republic of China Hubei Exit Inspection and Quarantine, Shenzhen wife Genetic Engineering Co., Ltd.

1 Scope

This standard specifies the EV71 virus in shellfish fluorescent RT-PCR detection method.

This standard is applicable to the detection of EV71 virus in shellfish.

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, 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

3 Terms and Definitions

The following terms and definitions apply to this document.

Intestinal virus EV71 Enterovirus71 Intestinal virus EV71, namely enterovirus type 71 (referred to EV71), belongs to the small RNA virus family, enterovirus, EV71 and Cox A16-type foot and mouth disease is the most common disease-causing viruses. Including enterovirus poliovirus (Poliovirus), Coxsackie virus (Coxasckievirus) A group 16,4,5,7,9,10 type and group B 2,5,13 type echovirus (ECHOviruses) and enterovirus 68,69,70,71,72 and

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other serotypes.

Fluorescent RT-PCR reai-timefluorescence RT-PCR Real-time quantitative RT-PCR method is based on the conventional RT-PCR, adding a specific fluorescent probe. The probe it is an oligonucleotide labeled at both ends of a report fluorophore and a quencher fluorophore. Probe is intact, the group sent a letter report no. absorbed quencher; PCR amplification, Taq polymerase 5'-3 'exonuclease activity of the enzyme degradation of the probe, so that the report fluorophore and quenching Separating fluorophore, fluorescence monitoring system is thus acceptable to the fluorescence signal, i.e., an amplification of each DNA strand, there is formed a fluorescent molecule, PCR products to achieve the accumulation and formation of the fluorescence signal completely synchronized.

Ct values cyclethreshold when the number of cycles the amount of fluorescence signal of each reaction tube reaches a set threshold experienced.

4 Summary

Glycine buffer on the food samples were homogenized by a high speed homogenizer physical means, after incubation promote the release of virus particles, Recycling PEG8000 settlement virus particles. Then using Trizol-reagent or other equivalent lysate extract total RNA precipitate, and Further use coated with Oligo (dT) magnetic beads with EV71 virus RNA3 25 'end of Poly (A) tail-specific binding, the purified virus

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