

ISO 15216-2:2019 (E)

Microbiology of the food chain — Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR — Part 2: Method for detection

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Annex A (normative) Diagram of procedure

Annex B (normative) Composition and preparation of reagents and buffers

B.1 5 × PEG/NaCl solution (500 g/l PEG 8 000, 1,5 mol/l NaCl)

B.1.1 Composition

B.1.2 Preparation

B.2 Chloroform/butanol mixture (1:1 volume fraction)

B.2.1 Composition

B.2.2 Preparation

B.3 Proteinase K solution (3000 U/l)

B.3.1 Composition

B.3.2 Preparation

B.4 Phosphate-buffered saline (PBS)

B.4.1 Composition

B.4.2 Preparation

B.5 Tris/glycine/beef extract (TGBE) buffer

B.5.1 Composition

B.5.2 Preparation

B.6 Tris solution (1 mol/l)

B.6.1 Composition

B.6.2 Preparation

B.7 EDTA solution (0,5 mol/l)

B.7.1 Composition

B.7.2 Preparation

B.8 Tris EDTA (TE) buffer (10 mmol/l Tris, 1 mmol/l EDTA)

B.8.1 Composition

B.8.2 Preparation

Annex C (informative) Real-time RT-PCR mastermixes and cycling parameters

Annex D (informative) Real-time RT-PCR primers and hydrolysis probes for the detection of HAV, norovirus GI and GII and mengo virus (process control)

D.1 HAV

D.2 Norovirus GI

D.3 Norovirus GII

D.4 Mengo virus

Annex E (informative) Growth of mengo virus strain MC0 for use as a process control

E.1 General

E.2 Reagents and apparatus

E.3 Procedure

Annex F (informative) RNA extraction using the BioMerieux NucliSens®3 3 BioMerieux NucliSens® is the trade name of a product supplied by BioMerieux. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results. system

F.1 Reagents

F.2 Apparatus

F.3 Procedure

Annex G (informative) Generation of external control RNA (EC RNA) stocks

G.1 General

- G.2 Reagents and apparatus**
- G.3 Linearization of plasmid DNA**
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Annex H (informative) Typical optical plate layout

Annex I (informative) Method validation studies and performance characteristics

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