

ISO/TS 15216-2:2013-03 (E)

Microbiology of food and animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 2: Method for qualitative detection

Contents	Page
Foreword	iv
Introduction	vii
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	3
4.1 Virus extraction	3
4.2 RNA extraction	3
4.3 Real-time reverse transcription polymerase chain reaction (real-time RT-PCR)	3
4.4 Control materials	4
4.5 Test results	4
5 Reagents	4
5.1 General	4
5.2 Reagents used as supplied	4
5.3 Prepared reagents	6
6 Apparatus and materials	6
7 Sampling	8
8 Procedure	8
8.1 General laboratory requirements	8
8.2 Virus extraction	8
8.3 RNA extraction	10
8.4 Real-time RT-PCR	10
9 Interpretation of results	12
9.1 General	12
9.2 Construction of process control virus RNA standard curve	12
9.3 Control for amplification efficiency	12
9.4 Calculation of extraction efficiency	13
9.5 Theoretical limit of detection	13
10 Expression of results	13
11 Test report	14
Annex A (normative) Diagram of procedure	15
Annex B (informative) Real-time RT-PCR mastermixes and cycling parameters	16
Annex C (informative) Real-time RT-PCR primers and hydrolysis probes for the detection of HAV, norovirus GI and GII and mengo virus (process control)	17

Annex D (informative) Growth of mengo virus strain MC0 for use as a process control	19
Annex E (informative) RNA extraction using the BioMerieux NucliSens® system	20
Annex F (normative) Composition and preparation of reagents and buffers	22
Annex G (informative) Generation of external control RNA (EC RNA) stocks	24
Annex H (informative) Typical optical plate layout	26
Bibliography	27