

DIN EN ISO 15216-2:2019-12 (Englisch)

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 (ISO 15216-2:2019)

Contents	Page
European foreword	4
Foreword	5
Introduction	6
1 Scope	7
2 Normative references	7
3 Terms and definitions	7
4 Principle	9
4.1 Virus extraction.....	9
4.2 RNA extraction.....	9
4.3 Real-time RT-PCR.....	9
4.4 Control materials.....	10
4.4.1 Process control virus.....	10
4.4.2 EC RNA control.....	10
4.5 Test results.....	10
5 Reagents	10
5.1 General.....	10
5.2 Reagents used as supplied.....	10
5.3 Reagents requiring preparation.....	12
6 Equipment and consumables	13
7 Sampling	14
8 Procedure	14
8.1 General laboratory requirements.....	14
8.2 Virus extraction.....	14
8.2.1 General.....	14
8.2.2 Process control virus material.....	15
8.2.3 Negative process control.....	15
8.2.4 Surfaces.....	15
8.2.5 Soft fruit and leaf, stem and bulb vegetables.....	15
8.2.6 Bottled water.....	16
8.2.7 Bivalve molluscan shellfish (BMS).....	16
8.3 RNA extraction.....	17
8.4 Real-time RT-PCR.....	17
8.4.1 General requirements.....	17
8.4.2 Real-time RT-PCR analysis.....	18
9 Interpretation of results	20
9.1 General.....	20
9.2 Construction of process control virus RNA standard curve.....	20
9.3 Control for RT-PCR inhibition.....	20
9.4 Calculation of extraction efficiency.....	21
10 Expression of results	21
11 Performance characteristics of the method	22
11.1 Validation study.....	22

11.2	Sensitivity	22
11.3	Specificity	22
11.4	LOD ₅₀	22
12	Test report	22
Annex A	(normative) Diagram of procedure	23
Annex B	(normative) Composition and preparation of reagents and buffers	24
Annex C	(informative) Real-time RT-PCR mastermixes and cycling parameters	27
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)	28
Annex E	(informative) Growth of mengo virus strain MC₀ for use as a process control	31
Annex F	(informative) RNA extraction using the BioMerieux NucliSens® system	32
Annex G	(informative) Generation of external control RNA (EC RNA) stocks	34
Annex H	(informative) Typical optical plate layout	37
Annex I	(informative) Method validation studies and performance characteristics	38
Bibliography	46