

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