

DIN EN ISO 15216-1:2021-10 (E)

Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR - Part 1: Method for quantification (ISO 15216-1:2017 + Amd 1:2021) (includes Amendment A1:2021)

| Contents | | Page |
|------------------------------------------------|--------------------------------------------------|-------------|
| European foreword | | 4 |
| [A1] European foreword to Amendment [A1] | | 5 |
| Foreword | | 6 |
| [A1] Foreword to Amendment [A1] | | 7 |
| Introduction | | 8 |
| 1 | Scope | 9 |
| 2 | Normative references | 9 |
| 3 | Terms and definitions | 9 |
| 4 | Principle | 11 |
| 4.1 | Virus extraction | 11 |
| 4.2 | RNA extraction | 12 |
| 4.3 | Real-time RT-PCR | 12 |
| 4.4 | Control materials | 12 |
| 4.4.1 | Process control virus | 12 |
| 4.4.2 | Double-stranded DNA (dsDNA) control | 12 |
| 4.4.3 | EC RNA control | 12 |
| 4.5 | Test results | 13 |
| 5 | Reagents | 13 |
| 5.1 | General | 13 |
| 5.2 | Reagents used as supplied | 13 |
| 5.3 | Prepared reagents | 14 |
| 6 | Equipment and consumables | 15 |
| 7 | Sampling | 17 |
| 8 | Procedure | 17 |
| 8.1 | General laboratory requirements | 17 |
| 8.2 | Virus extraction | 17 |
| 8.2.1 | Process control virus material | 17 |
| 8.2.2 | Negative process control | 17 |
| 8.2.3 | Food surfaces | 17 |
| 8.2.4 | Soft fruit, leaf, stem and bulb vegetables | 17 |
| 8.2.5 | Bottled water | 18 |
| 8.2.6 | Bivalve molluscan shellfish | 19 |
| 8.3 | RNA extraction | 19 |
| 8.4 | Real-time RT-PCR | 20 |
| 8.4.1 | General requirements | 20 |
| 8.4.2 | Real-time RT-PCR analysis | 20 |
| 9 | Interpretation of results | 22 |
| 9.1 | General | 22 |
| 9.2 | Construction of standard curves | 22 |
| 9.3 | Calculation of RT-PCR inhibition | 23 |
| 9.4 | Calculation of extraction efficiency | 23 |
| 9.5 | Sample quantification | 24 |

| | | |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 10 | Expression of results | 25 |
| 11 | Precision | 25 |
| | 11.1 Interlaboratory study..... | 25 |
| | 11.2 Repeatability..... | 25 |
| | 11.3 Reproducibility limit..... | 26 |
| 12 | Test report | 26 |
| | Annex A (normative) Diagram of procedure | 27 |
| | Annex B (normative) Composition and preparation of reagents and buffers | 28 |
| | Annex C (informative) Real-time RT-PCR mastermixes and cycling parameters | 31 |
| | 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) | 32 |
| | Annex E (informative) Growth of mengo virus strain MC₀ for use as a process control | 34 |
| | Annex F (informative) RNA extraction using the NucliSENS® system | 35 |
| | Annex G (informative) Generation of dsDNA control stocks | 37 |
| | Annex H (informative) Generation of EC RNA stocks | 40 |
| | Annex I (informative) Typical optical plate layout | 42 |
| | Annex J (informative) Method validation studies and performance characteristics | 43 |
| | Bibliography | 54 |