

ISO 15216-1:2017-03 (E)

Microbiology of the food chain - Horizontal method for determination of hepa titis A virus and norovirus using real-time RT-PCR - Part 1: Method for quantification

| Contents | | Page |
|--------------------|--|-------------|
| Foreword | | v |
| Introduction | | vi |
| 1 | Scope | 1 |
| 2 | Normative references | 1 |
| 3 | Terms and definitions | 1 |
| 4 | Principle | 3 |
| 4.1 | Virus extraction | 3 |
| 4.2 | RNA extraction | 4 |
| 4.3 | Real-time RT-PCR | 4 |
| 4.4 | Control materials | 4 |
| 4.4.1 | Process control virus | 4 |
| 4.4.2 | Double-stranded DNA (dsDNA) control | 4 |
| 4.4.3 | EC RNA control | 4 |
| 4.5 | Test results | 5 |
| 5 | Reagents | 5 |
| 5.1 | General | 5 |
| 5.2 | Reagents used as supplied | 5 |
| 5.3 | Prepared reagents | 6 |
| 6 | Equipment and consumables | 7 |
| 7 | Sampling | 9 |
| 8 | Procedure | 9 |
| 8.1 | General laboratory requirements | 9 |
| 8.2 | Virus extraction | 9 |
| 8.2.1 | Process control virus material | 9 |
| 8.2.2 | Negative process control | 9 |
| 8.2.3 | Food surfaces | 9 |
| 8.2.4 | Soft fruit, leaf, stem and bulb vegetables | 9 |
| 8.2.5 | Bottled water | 10 |
| 8.2.6 | Bivalve molluscan shellfish | 11 |
| 8.3 | RNA extraction | 11 |
| 8.4 | Real-time RT-PCR | 12 |
| 8.4.1 | General requirements | 12 |
| 8.4.2 | Real-time RT-PCR analysis | 12 |
| 9 | Interpretation of results | 14 |
| 9.1 | General | 14 |
| 9.2 | Construction of standard curves | 14 |
| 9.3 | Calculation of RT-PCR inhibition | 15 |
| 9.4 | Calculation of extraction efficiency | 15 |
| 9.5 | Sample quantification | 16 |
| 10 | Expression of results | 17 |

| | | |
|--|------------------------------------|-----------|
| 11 | Precision | 17 |
| 11.1 | Interlaboratory study | 17 |
| 11.2 | Repeatability | 17 |
| 11.3 | Reproducibility limit | 18 |
| 12 | Test report | 18 |
| Annex A (normative) Diagram of procedure | | 19 |
| Annex B (normative) Composition and preparation of reagents and buffers | | 20 |
| Annex C (informative) Real-time RT-PCR mastermixes and cycling parameters | | 23 |
| 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) | | 24 |
| Annex E (informative) Growth of mengo virus strain MC0 for use as a process control | | 27 |
| Annex F (informative) RNA extraction using the NucliSENS® system | | 28 |
| Annex G (informative) Generation of dsDNA control stocks | | 30 |
| Annex H (informative) Generation of EC RNA stocks | | 33 |
| Annex I (informative) Typical optical plate layout | | 35 |
| Annex J (informative) Method validation studies and performance characteristics | | 37 |
| Bibliography | | 48 |