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S.R. CEN ISO/TS 15216-1:2013

Microbiology of food and animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 1: Method for quantification (ISO/TS 15216-1:2013, Corrected Version 2013-05-01)

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TECHNICAL SPECIFICATION

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CEN ISO/TS 15216-1

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English Version

Microbiology of food and animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 1: Method for quantification (ISO/TS 15216-1:2013, Corrected Version 2013-05-01)

Microbiologie des aliments - Méthode horizontale pour la recherche des virus de l'hépatite A et norovirus dans les aliments par la technique RT-PCR en temps réel - Partie 1: Méthode de quantification (ISO/TS 15216-1:2013, Version Corrigée 2013-05-01)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zum Nachweis von Hepatitis A-Viren und Noroviren in Lebensmitteln mittels Real time PCR - Teil 1: Verfahren zur quantitativen Bestimmung (ISO/TS 15216-1:2013, korrigierten Fassung von 2013-05-01)

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Foreword

This document (CEN ISO/TS 15216-1:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 “Food analysis - Horizontal methods” the secretariat of which is held by DIN.

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TECHNICAL SPECIFICATION

ISO/TS 15216-1

First edition
2013-03-15

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2013-05-01

Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR —

Part 1: Method for quantification

Microbiologie des aliments — Méthode horizontale pour la recherche des virus de l'hépatite A et norovirus dans les aliments par la technique RT-PCR en temps réel —

Partie 1: Méthode de quantification

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 15216-1 was prepared by the European Committee for Standardization (CEN), in collaboration with Technical committee ISO/TC 34, *Food products*, Subcommittee SC 9 *Microbiology*.

This corrected version of ISO/TS 15216-1:2013 incorporates the following corrections.

— Throughout, textual references have been updated to take reordering of the annexes into account. Annex B was formerly Annex E; Annex C was formerly Annex D; Annex D was formerly Annex G; Annex E was formerly Annex C; Annex F was formerly Annex B; Annex G was formerly Annex H; Annex H was formerly Annex I; Annex I was formerly Annex F.

— Many cross-references to reagents or apparatus subclauses are added.

— Where units of shaking operations are mentioned, “oscillations min^{-1}” replaces “min^{-1}”.

— A phrase citing Annex A is added to the end of the introduction.

— The definitions for “food surface” (formerly 3.2 and 3.3) are combined and expanded in a redrafted 3.2; in consequence, the following terms in Clause 3 are renumbered.

— In 3.4, Note 2, “There is only one serotype” is transposed to the end of Note 1. Also, “group 2 biological agent by the European Union and as a risk group 2 human aetiological agent by the United States National Institutes of Health” replaces “UK Advisory Committee on Dangerous Pathogens (ACDP) hazard group 2 pathogen”.

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— In 3.5, Note 2, "group 2 biological agents by the European Union and as risk group 2 human aetiological agents by the United States National Institutes of Health" replaces "ACDP hazard group 2 pathogens".

— In 3.6 and 3.7, "estimation of number of copies" replaces "quantification".

— In 3.13, "used in" replaces "used as template in".

— In 5.2.11, "from *Aspergillus niger* or *A. aculeatus"* is inserted after "Pectinase".

— In 6.1,"Aerosol resistant tips should be used unless unobstructed tips are required, e.g. for aspiration." is inserted.

— In 6.5, "37 ± 1,0" replaces "37 ± 10".

— A redrafted 6.10 on centrifuge(s) and rotor(s) replaces the former 6.10 and 6.11, with consequent renumbering of the following subclauses.

— In 6.19, the square brackets are deleted.

— In 6.27, "**Real-time PCR machine(s)**, i.e. thermal cycler(s)," replaces "**Thermal cycler(s)**".

— In 6.28, "selected real-time PCR" replaces "selected PCR".

— In 8.1, "Samples arriving already frozen should be defrosted prior to testing." is inserted as the second sentence.

— 8.2.3 Is redrafted.

— In 8.2.4, paragraph 2,"buffer (5.3.5) (for soft fruit samples, add 30 units pectinase from A. niger, or 1 140 units pectinase from A. aculeatus to the buffer) and" replaces "buffer (for soft fruit samples, add 30 units pectinase to the buffer) and".

— In 8.2.6, paragraph 2, "and the animal is supported with a rubber block" is added.

— In 8.2.6, last paragraph, "min at room temperature, decant" replaces "min, decant"

— In 8.4.2.3, paragraph 1,"using a real-time PCR machine (6.27)" is added.

— In 9.3, Note 1, "For a dsDNA standard curve with an idealized slope of −3,32, if the C_q value of the sample RNA + EC RNA well is <2,00 greater than the C_q value of the water + EC RNA well, the amplification efficiency is >25 % and therefore acceptable; if the C_q value of the sample RNA + EC RNA well is >2,00 greater than the C_q value of the water + EC RNA well, the amplification efficiency is <25% and therefore not acceptable." is added.

— In 9.4, Note 1 "a process control virus recovery (equal to the extraction efficiency in matrices other than BMS) of 100 %. For a process control virus RNA standard curve with an idealized slope of −3,32, if the C_q value of an undiluted sample RNA well is <6,64 greater than the C_q value of the undiluted process control virus RNA, the process control virus recovery for that sample is >1% and therefore acceptable" replaces "an extraction efficiency of 100 %".

— The title of Annex B has been expanded to read, "Real-time RT-PCR mastermixes and cycling parameters".

— In Table B.1, footnote a, "real-time PCR machines" twice replaces "real-time machines".

— In C.1, "This primer set amplifies a product of 173 bp corresponding to nucleotides 68–240 of HAV isolate HM174 43c (GenBank accession number M59809)." is added as paragraph 2.

— In C.2, "This primer set amplifies a product of 86 bp corresponding to nucleotides 5291–5376 of Norwalk virus (GenBank accession number M87661)." is added as paragraph 2."

— In C.3, "This primer set amplifies a product of 89 bp corresponding to nucleotides 5012–5100 of Lordsdale virus (GenBank accession number X86557)." is added as paragraph 2."

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— In C.4, “This primer set amplifies a product of 100 bp corresponding to nucleotides 110–209 of the deletant mengo virus strain MC0 used in the development of this part of ISO/TS 15216. This corresponds to nucleotides 110–270 of the non-deletant mengo virus isolate M (GenBank accession number L22089).” is added as paragraph 2.”

— In H.5, “mastermix (if the C_q difference between EC RNA stock tested with heat-treated and untreated mastermix is <10 for a dsDNA standard curve with an idealized slope of −3,32), the” replaces “mastermix, the”.

ISO/TS 15216 consists of the following parts, under the general title *Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR*:

— *Part 1: Method for quantification*

— *Part 2: Method for qualitative detection*

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Introduction

Hepatitis A virus (HAV) and norovirus (NoV) are important agents of food-borne human viral illness. No routine methods exist to culture these viruses from food matrices. Detection is therefore reliant on molecular methods using the reverse-transcriptase polymerase chain reaction (RT-PCR). As many food matrices contain substances that are inhibitory to RT-PCR, it is necessary to use an extraction method that produces highly clean RNA preparations that are fit for purpose. For food surfaces, viruses are removed by swabbing. For soft fruit and salad vegetables, virus extraction is by elution with agitation followed by precipitation with PEG/NaCl. For bottled water, adsorption and elution using positively charged membranes followed by concentration by ultrafiltration is used and for bivalve molluscan shellfish, viruses are extracted from the tissues of the digestive glands using treatment with a proteinase K solution. For all matrices which are not covered by this Technical Specification, it is necessary to validate this method. All matrices share a common RNA extraction method based on virus capsid disruption with chaotropic reagents followed by adsorption of RNA to silica particles. Real-time RT-PCR monitors amplification throughout the PCR cycle by measuring the excitation of fluorescently labelled molecules. In the 5' fluorogenic nuclease real-time RT-PCR assay, the fluorescent labels are attached to a sequence-specific nucleotide probe (hydrolysis probe) that also enables simultaneous confirmation of target template. These modifications increase the sensitivity and specificity of the PCR method, and obviate the need for additional amplification product confirmation steps post PCR. Due to the complexity of the method, it is necessary to include a comprehensive suite of controls. The method described in this part of ISO/TS 15216 enables quantification of levels of virus RNA in the test sample. A schematic diagram of the testing procedure is shown in Annex A.

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Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR —

Part 1: Method for quantification

1 Scope

This part of ISO/TS 15216 describes a method for quantification of levels of HAV and NoV genogroup I (GI) and II (GII) RNA, from test samples of foodstuffs or food surfaces. Following liberation of viruses from the test sample, viral RNA is then extracted by lysis with guanidine thiocyanate and adsorption on silica. Target sequences within the viral RNA are amplified and detected by real-time RT-PCR.

This approach is also relevant for detection of the target viruses on fomites, or of other human viruses in foodstuffs, on food surfaces or on fomites following appropriate validation and using target-specific primer and probe sets.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22174 and the following apply.

3.1
foodstuff
substance used or prepared for use as food

Note 1 to entry: For the purposes of this part of ISO/TS 15216, this definition includes bottled water.

3.2
food surface
surface of food, food preparation surface or food contact surface

3.3
fomite
inanimate object or material on which infectious agents can be conveyed

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