SLOVENSKI STANDARD
SIST-TS CEN ISO/TS 15216-2:2013

01-september-2013

Mikrobiologija živil in krme - Horizontalna metoda za ugotavljanje virusa hepatitisa A in norovirusov v živilih z RT-PCR v realnem času - 2. del: Kvalitativna metoda (ISO/TS 15216-2:2013, popravljena verzija 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 2: Method for qualitative detection (ISO/TS 15216-2:2013, Corrected Version 2013-05-01)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zur Bestimmung von Hepatitis A-Viren und Noroviren in Lebensmitteln mittels Real-time-RT-PCR - Teil 2: Verfahren für den qualitativen Nachweis (ISO/TS 15216-2:2013)

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 2: Méthode de détection qualitative (ISO/TS 15216-2:2013, Version Corrigée 2013 -05-01)

Ta slovenski standard je istoveten z: **CEN ISO/TS 15216-2:2013**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

SIST-TS CEN ISO/TS 15216-2:2013 **en**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN ISO/TS 15216-2

May 2013

ICS 07.100.30

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 2: Method for qualitative detection (ISO/TS 15216-2:2013, 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 2: Méthode de détection qualitative (ISO/TS 15216-2:2013, 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 2: Verfahren für den qualitativen Nachweis (ISO/TS 15216-2:2013, korrigierten Fassung von 2013-05-01)

This Technical Specification (CEN/TS) was approved by CEN on 8 March 2013 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. CEN ISO/TS 15216-2:2013: E

Contents

Page

Foreword

This document (CEN ISO/TS 15216-2:2013) 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.

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

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO/TS 15216-2:2013, Corrected Version 2013-05-01 has been approved by CEN as CEN ISO/TS 15216-2:2013 without any modification.

TECHNICAL SPECIFICATION

ISO/TS 15216-2

First edition
2013-03-15

Corrected version
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 2: Method for qualitative detection

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 2: Méthode de détection qualitative

Reference number
ISO/TS 15216-2:2013(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

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 15216-2 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 15216-2:2013 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 F.

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

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

— 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".

— 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 3.13, “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 9.4, Note 1 “has 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 “will have 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.

— In C.4, “This primer set amplifies a product of 100 bp corresponding to nucleotides 110–209 of the deletant mengo virus strain MC_0 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 G.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*

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 qualitative detection of virus RNA in the test sample. A schematic diagram of the testing procedure is shown in Annex A.