PD CEN ISO/TS 15216-2:2013

BSI Standards Publication

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

bsi.

...making excellence a habit.™

Document Sharing Hub

Full document details: PD CEN ISO/TS 15216-2:2013

National foreword

This Published Document is the UK implementation of CEN ISO/TS 15216-2:2013.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2013. Published by BSI Standards Limited 2013

ISBN 978 0 580 80627 8

ICS 07.100.30

Compliance with a British Standard cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 May 2013.

Amendments issued since publication

Date	Text affected

Documents
Document Sharing Hub

Full document details: PD CEN ISO/TS 15216-2:2013

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

© 2013 CEN All rights of exploitation in any form and by any means reserved worldwide for CEN national Members.

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

Documents
Document Sharing Hub

Full document details: PD CEN ISO/TS 15216-2:2013

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.

Documents
Document Sharing Hub

Full document details: PD CEN ISO/TS 15216-2:2013

Contents

Page

Foreword ... **iv**
Introduction ... **vii**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **1**
4 Principle ... **3**
4.1 Virus extraction ... 3
4.2 RNA extraction ... 3
4.3 Real-time reverse transcription polymerase chain reaction (real-time RT-PCR) ... 3
4.4 Control materials ... 4
4.5 Test results ... 4
5 Reagents ... **4**
5.1 General ... 4
5.2 Reagents used as supplied ... 4
5.3 Prepared reagents ... 6
6 Apparatus and materials ... **6**
7 Sampling ... **8**
8 Procedure ... **8**
8.1 General laboratory requirements ... 8
8.2 Virus extraction ... 8
8.3 RNA extraction ... 10
8.4 Real-time RT-PCR ... 10
9 Interpretation of results ... **12**
9.1 General ... 12
9.2 Construction of process control virus RNA standard curve ... 12
9.3 Control for amplification efficiency ... 12
9.4 Calculation of extraction efficiency ... 13
9.5 Theoretical limit of detection ... 13
10 Expression of results ... **13**
11 Test report ... **14**
Annex A (normative) **Diagram of procedure** ... **15**
Annex B (informative) **Real-time RT-PCR mastermixes and cycling parameters** ... **16**
Annex C (informative) **Real-time RT-PCR primers and hydrolysis probes for the detection of HAV, norovirus GI and GII and mengo virus (process control)** ... **17**
Annex D (informative) **Growth of mengo virus strain MC_0 for use as a process control** ... **19**
Annex E (informative) **RNA extraction using the BioMerieux NucliSens® system** ... **20**
Annex F (normative) **Composition and preparation of reagents and buffers** ... **22**
Annex G (informative) **Generation of external control RNA (EC RNA) stocks** ... **24**
Annex H (informative) **Typical optical plate layout** ... **26**
Bibliography ... **27**

© ISO 2013 – All rights reserved

Documents
Document Sharing Hub
Full document details: PD CEN ISO/TS 15216-2:2013

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”.

© ISO 2013 – All rights reserved
Documents
Document Sharing Hub
Full document details: PD CEN ISO/TS 15216-2:2013

— 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.

© ISO 2013 – All rights reserved

Documents
Document Sharing Hub
Full document details: PD CEN ISO/TS 15216-2:2013

— 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*

© ISO 2013 – All rights reserved
Documents
Document Sharing Hub
Full document details: PD CEN ISO/TS 15216-2:2013

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.

© ISO 2013 – All rights reserved

Documents
Document Sharing Hub
Full document details: PD CEN ISO/TS 15216-2:2013

Documents
Document Sharing Hub

Full document details: PD CEN ISO/TS 15216-2:2013

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

Part 2: Method for qualitative detection

1 Scope

This part of ISO/TS 15216 describes a method for qualitative detection of HAV and NoV genogroups I (GI) and II (GII), 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

© ISO 2013 – All rights reserved

Documents
Document Sharing Hub

Full document details: PD CEN ISO/TS 15216-2:2013

3.4
hepatitis A virus
HAV
member of the *Picornaviridae* family responsible for infectious hepatitis

Note 1 to entry: Genetically, HAV can be subdivided into six genotypes on the basis of the VP1/2A region (genotypes 1, 2, and 3 have been found in humans, while genotypes 4, 5, and 6 are of simian origin). There is only one serotype.

Note 2 to entry: Transmission occurs via the faecal-oral route by person-to-person contact, through the consumption of contaminated foodstuffs, contact with contaminated water or food surfaces, or contact with contaminated fomites. Hepatitis A virus is classified as a 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.

3.5
norovirus
member of the *Caliciviridae* family responsible for sporadic cases and outbreaks of acute gastroenteritis

Note 1 to entry: Genetically, norovirus can be subdivided into five separate genogroups.

Note 2 to entry: Three of these genogroups, GI, GII and GIV have been implicated in human gastrointestinal disease. GI and GII are responsible for the vast majority of clinical cases. Transmission occurs via the faecal-oral route by person-to-person contact, through the consumption of contaminated foodstuffs or through contact with contaminated water or food surfaces or contact with contaminated fomites. Genogroup I and II noroviruses are classified as group 2 biological agents by the European Union and as risk group 2 human aetiological agents by the United States National Institutes of Health.

3.6
detection of hepatitis A virus
detection of HAV RNA in a predetermined mass or volume of foodstuff, or area of food surface

3.7
detection of norovirus
detection of norovirus RNA in a predetermined mass or volume of foodstuff, or area of food surface

3.8
process control virus
virus added to the sample portion at the earliest opportunity prior to virus extraction to control for extraction efficiency

3.9
process control virus RNA
RNA released from the process control virus in order to produce standard curve data for the estimation of extraction efficiency

3.10
negative RNA extraction control
control free of target RNA carried through all steps of the RNA extraction and detection procedure to monitor any cross-contamination events

3.11
negative process control
target pathogen-free sample of the food matrix which is run through all stages of the analytical process

3.12
hydrolysis probe
fluorescent probe coupled with two fluorescent molecules which are sterically separated by the 5′-3′-exonuclease activity of the enzyme during the amplification process

© ISO 2013 – All rights reserved
Documents
Document Sharing Hub
Full document details: PD CEN ISO/TS 15216-2:2013