BS EN ISO 15216-1:2017

BSI Standards Publication

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)

bsi.

This British Standard is the UK implementation of EN ISO 15216-1:2017. It supersedes PD CEN ISO/TS 15216-1:2013 which is withdrawn.

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.

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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)

Microbiologie dans la chaine alimentaire - Méthode horizontale pour la recherche des virus de l'hépatite A et norovirus par la technique RT-PCR en temps réel - Partie 1: Méthode de quantification (ISO 15216-1:2017)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zur Bestimmung von Hepatitis A-Virus und Norovirus mittels Real-time-RT-PCR - Teil 1: Verfahren zur Quantifizierung (ISO 15216-1:2017)

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CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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European foreword

This document (EN ISO 15216-1:2017) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2017 and conflicting national standards shall be withdrawn at the latest by September 2017.

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.

This document supersedes CEN ISO/TS 15216-1:2013.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: 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, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 15216-1:2017 has been approved by CEN as EN ISO 15216-1:2017 without any modification.

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, in collaboration with ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition cancels and replaces ISO/TS 15216-1:2013, which has been technically revised with the following changes:

— use of linear dsDNA molecules for quantification prescribed;

— use of a suitable buffer for dilution of control materials prescribed;

— change to the method for generating process control virus RNA for the standard curve;

— addition of breakpoints with defined temperature and time parameters in the extraction methods;

— change in terminology from amplification efficiency to RT-PCR inhibition;

— addition of extra real-time RT-PCR reactions for negative controls;

— addition of precision data and results of interlaboratory study.

A list of all parts in the ISO 15216 series can be found on the ISO website.

Introduction

Hepatitis A virus (HAV) and norovirus are important agents of food-borne human viral illness. No routine methods exist for culture of norovirus, and HAV culture methods are not appropriate for routine application to 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, leaf, stem and bulb 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 (BMS), viruses are extracted from the tissues of the digestive glands using treatment with a proteinase K solution. For all matrices that are not covered by this document, 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 real-time RT-PCR cycle by measuring the excitation of fluorescently labelled molecules. In real-time RT-PCR with hydrolysis probes, the fluorescent label is attached to a sequence-specific nucleotide probe that also enables simultaneous confirmation of target template. These modifications increase the sensitivity and specificity of the real-time RT-PCR method, and obviate the need for additional amplification product confirmation steps post real-time RT-PCR. Due to the complexity of the method, it is necessary to include a comprehensive suite of controls. The method described in this document enables quantification of levels of virus RNA in the test sample. A schematic diagram of the testing procedure is shown in Annex A.

The main changes, listed in the Foreword, introduced in this document compared to ISO/TS 15216-1:2013 are considered as minor (see ISO 17468).

INTERNATIONAL STANDARD

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

1 Scope

This document specifies a method for the quantification of levels of HAV and norovirus genogroup I (GI) and II (GII) RNA, from test samples of foodstuffs (soft fruit, leaf, stem and bulb vegetables, bottled water, BMS) 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 method is not validated for detection of the target viruses in other foodstuffs (including multi-component foodstuffs), or any other matrices, nor for the detection of other viruses in foodstuffs, food surfaces or other matrices.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 20838, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for amplification and detection for qualitative methods*

ISO 22119, *Microbiology of food and animal feeding stuffs — Real-time polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

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, ISO 22119 and ISO 20838 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
foodstuff
substance used or prepared for use as food

Note 1 to entry: For the purposes of this document, this definition includes bottled water.