SLOVENSKI STANDARD
SIST EN ISO 15216-2:2019

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Nadomešča:
SIST-TS CEN ISO/TS 15216-2:2013

Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje virusa hepatitisa A in norovirusov z RT-PCR v realnem času - 2. del: Metoda za ugotavljanje (ISO 15216-2:2019)

Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR - Part 2: Method for detection (ISO 15216-2:2019)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zur Bestimmung von Hepatitis A-Virus und Norovirus in Lebensmitteln mittels Real-time-RT-PCR - Teil 2: Nachweisverfahren (ISO 15216-2:2019)

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 2: Méthode de détection (ISO 15216-2:2019)

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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 2: Method for detection (ISO 15216-2:2019)

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European foreword

This document (EN ISO 15216-2:2019) 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.

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of an identical text or by endorsement, at the latest by March 2020, and conflicting national standards shall be withdrawn at the latest by March 2020.

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

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

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Endorsement notice

The text of ISO 15216-2:2019 has been approved by CEN as EN ISO 15216-2:2019 without any modification.

INTERNATIONAL STANDARD

ISO 15216-2

First edition
2019-07

Microbiology of the food chain — Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR —

Part 2: Method for detection

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 2: Méthode de détection

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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.

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 of 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 www.iso.org/iso/foreword.html.

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee 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 15216-2:2013, which has been technically revised with the following changes:

— a requirement to use a suitable buffer for the dilution of control materials has been added;

— the method for generating process control virus RNA for the standard curve has been changed;

— breakpoints with a defined temperature and time parameters in the extraction methods have been added;

— the terminology has been changed from amplification efficiency to RT-PCR inhibition;

— extra real-time RT-PCR reactions for sample RNA and negative controls have been added;

— method characteristics and the results of method validation studies have been added.

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 surfaces, viruses are removed by swabbing. For soft fruit and 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. 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 detection 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 15216-2:2013, are considered as minor (see ISO 17468).

Microbiology of the food chain — Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR —

Part 2: Method for detection

1 Scope

This document specifies a method for detection of hepatitis A virus (HAV) and norovirus genogroups I (GI) and II (GII), from test samples of foodstuffs [(soft fruit, leaf, stem and bulb vegetables, bottled water, bivalve molluscan shellfish (BMS)] or surfaces using 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, 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 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 20838, ISO 22119, ISO 22174 and the following apply.

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

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

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

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.

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

3.3
soft fruit
small edible stoneless fruit

EXAMPLE Strawberries, raspberries, currants.

3.4
leaf, stem and bulb vegetables
leaves, stems and bulbs of plants, eaten as a vegetable

EXAMPLE Lettuce, green onions.

3.5
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* (3.1), contact with contaminated water or *surfaces* (3.2), or contact with contaminated fomites. HAV 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.

3.6
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 seven separate genogroups. 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.

Note 2 to entry: Transmission occurs via the faecal-oral route by person-to-person contact, through the consumption of contaminated *foodstuffs* (3.1), through contact with contaminated water or *surfaces* (3.2), or contact with contaminated fomites. GI and GII 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.

3.7
detection of HAV
detection of *HAV* (3.5) RNA in a predetermined mass or volume of *foodstuff* (3.1), or on the area of a *surface* (3.2)

3.8
detection of norovirus
detection of *norovirus* (3.6) RNA in a predetermined mass or volume of *foodstuff* (3.1), or on the area of a *surface* (3.2)

3.9
process control virus
virus added to the sample portion at the earliest opportunity prior to virus extraction to control for extraction efficiency

3.10
process control virus RNA
RNA extracted from the *process control virus* (3.9) in order to produce standard curve data for the estimation of extraction efficiency

3.11
negative RNA extraction control
control free of target RNA carried through all steps of the RNA extraction and detection procedure to monitor any contamination events

3.12
negative process control
target pathogen-free sample of the food matrix, or target pathogen-free non-matrix sample, that is run through all stages of the analytical process

3.13
hydrolysis probe
fluorescent probe coupled with a fluorescent reporter molecule and a quencher molecule, which are sterically separated by the 5′-3′-exonuclease activity of the enzyme during the amplification process

3.14
negative real-time RT-PCR control
aliquot of highly pure water used in a real-time RT-PCR reaction to assess contamination in the real-time RT-PCR reagents

3.15
external control RNA
EC RNA
reference RNA that can be used to assess inhibition of amplification for the real-time RT-PCR assay of relevance by being added in a defined amount to an aliquot of sample RNA in a separate reaction

EXAMPLE RNA synthesized by *in vitro* transcription from a plasmid carrying a copy of the target gene.

3.16
C_q value
quantification cycle, which is the cycle at which the target is quantified in a given real-time RT-PCR reaction

Note 1 to entry: This corresponds to the cycle at which reaction fluorescence rises above a threshold level.

4 Principle

4.1 Virus extraction

The foodstuffs and surfaces covered by this document are often highly complex matrices and the target viruses can be present at low concentrations. It is therefore necessary to carry out matrix-specific virus extraction and/or concentration in order to provide a substrate for subsequent common parts of the process. The choice of method depends upon the matrix.

4.2 RNA extraction

It is necessary to extract RNA using a method that yields RNA preparations of suitable purity to reduce the effect of RT-PCR inhibitors. In this document, the chaotropic agent guanidine thiocyanate is used to disrupt the viral capsid. RNA is then adsorbed to silica to assist purification through several washing stages. Purified viral RNA is released from the silica into a buffer prior to real-time RT-PCR.

4.3 Real-time RT-PCR

This document uses one-step real-time RT-PCR using hydrolysis probes. In one-step real-time RT-PCR, reverse transcription and PCR amplification are carried out consecutively in the same tube.

Real-time RT-PCR using hydrolysis probes utilizes a short DNA probe with a fluorescent label and a fluorescence quencher attached at the 5’ and 3’ ends, respectively. The assay chemistry ensures that as the quantity of amplified product increases, the probe is hydrolysed and the fluorescent signal from the label increases proportionately.

Due to the low levels of virus template often present in foodstuffs or surfaces and the strain diversity in the target viruses, the selection of fit-for-purpose one step real-time RT-PCR reagents and PCR primers and hydrolysis probes for the target viruses is important. Guidelines for their selection are given in