SLOVENSKI STANDARD
oSIST prEN ISO 15216-2:2018

01-julij-2018

Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje virusa hepatitisa A in norovirusov v živilih z RT-PCR v realnem času - 2. del: Metoda za ugotavljanje (ISO/DIS 15216-2:2018)

Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR - Part 2: Method for detection (ISO/DIS 15216-2:2018)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zur Bestimmung von Hepatitis A-Virus und Norovirus in Lebensmitteln mittels Real-time-RT-PCR - Teil 2: Nachweisverfahren (ISO/DIS 15216-2:2018)

Microbiologie de la chaine alimentaire - Méthode horizontale pour la recherche des virus de l'hépatite A et norovirus par RT-PCR en temps réel - Partie 2: Méthode de détection (ISO/DIS 15216-2:2018)

Ta slovenski standard je istoveten z: **prEN ISO 15216-2**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

oSIST prEN ISO 15216-2:2018 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 15216-2

ISO/TC **34**/SC **9** Secretariat: **AFNOR**

Voting begins on:
2018-04-30

Voting terminates on:
2018-07-23

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

Part 2:
Method for 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

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 15216-2:2018(E)

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Published in Switzerland

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Foreword

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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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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 Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition cancels and replaces ISO/TS 15216-2:2013, which has been technically revised with the following changes:

- 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 sample RNA and negative controls;
- addition of method characteristics and results of method validation studies.

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 International Standard, 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 part of ISO 15216 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[2]).

