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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)

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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.

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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.

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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.

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

<1> surface of food

3.3

food surface

<2> food preparation surface

3.4

fomite

inanimate object or material on which infectious agents can be conveyed