



NSAI
Standards

Irish Standard Recommendation
S.R. EN 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)

S.R. EN ISO 15216-2:2019

Incorporating amendments/corrigenda/National Annexes issued since publication:

The National Standards Authority of Ireland (NSAI) produces the following categories of formal documents:

I.S. xxx: Irish Standard — national specification based on the consensus of an expert panel and subject to public consultation.

S.R. xxx: Standard Recommendation — recommendation based on the consensus of an expert panel and subject to public consultation.

SWiFT xxx: A rapidly developed recommendatory document based on the consensus of the participants of an NSAI workshop.

This document replaces/revises/consolidates the NSAI adoption of the document(s) indicated on the CEN/CENELEC cover/Foreword and the following National document(s):

NOTE: The date of any NSAI previous adoption may not match the date of its original CEN/CENELEC document.

This document is based on:

EN ISO 15216-2:2019

Published:

2019-09-18

*This document was published
under the authority of the NSAI
and comes into effect on:*

2019-10-07

ICS number:

07.100.30

NOTE: If blank see CEN/CENELEC cover page

NSAI
1 Swift Square,
Northwood, Santry
Dublin 9

T +353 1 807 3800
F +353 1 807 3838
E standards@nsai.ie
W NSAI.ie

Sales:
T +353 1 857 6730
F +353 1 857 6729
W standards.ie

Údarás um Chaighdeáin Náisiúnta na hÉireann

National Foreword

S.R. EN ISO 15216-2:2019 is the adopted Irish version of the European Document EN 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)

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

For relationships with other publications refer to the NSAI web store.

Compliance with this document does not of itself confer immunity from legal obligations.

In line with international standards practice the decimal point is shown as a comma (,) throughout this document.

This page is intentionally left blank

EUROPEAN STANDARD

EN ISO 15216-2

NORME EUROPÉENNE

EUROPÄISCHE NORM

September 2019

ICS 07.100.30

Supersedes CEN ISO/TS 15216-2:2013

English Version

**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)**

Microbiologie dans la chaîne 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)

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)

This European Standard was approved by CEN on 27 July 2019.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

EN ISO 15216-2:2019 (E)

Contents	Page
European foreword.....	3

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.

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, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

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

This page is intentionally left blank

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 chaîne 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



Reference number
ISO 15216-2:2019(E)

© ISO 2019

ISO 15216-2:2019(E)



COPYRIGHT PROTECTED DOCUMENT

© ISO 2019

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

	Page
Foreword	v
Introduction	vi
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 RT-PCR.....	3
4.4 Control materials.....	4
4.4.1 Process control virus.....	4
4.4.2 EC RNA control.....	4
4.5 Test results.....	4
5 Reagents	4
5.1 General.....	4
5.2 Reagents used as supplied.....	4
5.3 Reagents requiring preparation.....	6
6 Equipment and consumables	7
7 Sampling	8
8 Procedure	8
8.1 General laboratory requirements.....	8
8.2 Virus extraction.....	8
8.2.1 General.....	8
8.2.2 Process control virus material.....	9
8.2.3 Negative process control.....	9
8.2.4 Surfaces.....	9
8.2.5 Soft fruit and leaf, stem and bulb vegetables.....	9
8.2.6 Bottled water.....	10
8.2.7 Bivalve molluscan shellfish (BMS).....	10
8.3 RNA extraction.....	11
8.4 Real-time RT-PCR.....	11
8.4.1 General requirements.....	11
8.4.2 Real-time RT-PCR analysis.....	12
9 Interpretation of results	14
9.1 General.....	14
9.2 Construction of process control virus RNA standard curve.....	14
9.3 Control for RT-PCR inhibition.....	14
9.4 Calculation of extraction efficiency.....	15
10 Expression of results	15
11 Performance characteristics of the method	16
11.1 Validation study.....	16
11.2 Sensitivity.....	16
11.3 Specificity.....	16
11.4 LOD ₅₀	16
12 Test report	16
Annex A (normative) Diagram of procedure	17
Annex B (normative) Composition and preparation of reagents and buffers	18
Annex C (informative) Real-time RT-PCR mastermixes and cycling parameters	21

ISO 15216-2:2019(E)

Annex D (informative) Real-time RT-PCR primers and hydrolysis probes for the detection of HAV, norovirus GI and GII and mengo virus (process control)	22
Annex E (informative) Growth of mengo virus strain MC₀ for use as a process control	25
Annex F (informative) RNA extraction using the BioMerieux NucliSens® system	26
Annex G (informative) Generation of external control RNA (EC RNA) stocks	28
Annex H (informative) Typical optical plate layout	31
Annex I (informative) Method validation studies and performance characteristics	32
Bibliography	40

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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.

ISO 15216-2:2019(E)**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

This is a free preview. Purchase the entire publication at the link below:

[Product Page](#)

-
- Looking for additional Standards? Visit Intertek Inform Infostore
 - Learn about LexConnect, All Jurisdictions, Standards referenced in Australian legislation
-