

Microbiology of the food chain - Method validation -
Part 2: Protocol for the validation of alternative
(proprietary) methods against a reference method (ISO
16140-2:2016)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 16140-2:2016 sisaldab Euroopa standardi EN ISO 16140-2:2016 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 16140-2:2016 consists of the English text of the European standard EN ISO 16140-2:2016.
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English Version

**Microbiology of the food chain - Method validation - Part 2:
Protocol for the validation of alternative (proprietary)
methods against a reference method (ISO 16140-2:2016)**

Microbiologie de la chaîne alimentaire - Validation des
méthodes - Partie 2: Protocole pour la validation de
méthodes alternatives (commerciales) par rapport à
une méthode de référence (ISO 16140-2:2016)

Mikrobiologie der Lebensmittelkette -
Verfahrensvalidierung - Teil 2: Arbeitsvorschrift für die
Validierung von alternativen (urheberrechtlich
geschützten) Verfahren anhand eines
Referenzverfahrens (ISO 16140-2:2016)

This European Standard was approved by CEN on 12 May 2016.

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

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

European foreword

This document (EN ISO 16140-2:2016) 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of an identical text or by endorsement, at the latest by January 2017, and conflicting national standards shall be withdrawn at the latest by January 2017.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 EN ISO 16140:2003.

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, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 16140-2:2016 has been approved by CEN as EN ISO 16140-2:2016 without any modification.

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 General principles for the validation of alternative methods	1
5 Qualitative methods — Technical protocol for validation	2
5.1 Method comparison study	2
5.1.1 General considerations	2
5.1.2 Paired or unpaired study	2
5.1.3 Sensitivity study	2
5.1.4 Relative level of detection study	7
5.1.5 Inclusivity and exclusivity study	9
5.2 Interlaboratory study	10
5.2.1 General considerations	10
5.2.2 Measurement protocol	10
5.2.3 Calculations and summary of data	12
5.2.4 Interpretation of data	15
6 Quantitative methods — Technical protocol for validation	16
6.1 Method comparison study	16
6.1.1 General considerations	16
6.1.2 Relative trueness study	16
6.1.3 Accuracy profile study	20
6.1.4 Limit of quantification study	24
6.1.5 Inclusivity and exclusivity study	24
6.2 Interlaboratory study	26
6.2.1 General considerations	26
6.2.2 Measurement protocol	26
6.2.3 Calculations, summary, and interpretation of data	27
Annex A (informative) Classification of sample types and suggested target combinations for validation studies	30
Annex B (normative) Order of preference for use of naturally and artificially contaminated samples in validation studies	46
Annex C (informative) General protocols for contamination by mixture and artificial contamination of foods	47
Annex D (informative) Models for RLOD calculations using data from the method comparison study	50
Annex E (normative) Points to be considered when selecting strains for testing inclusivity and exclusivity	52
Annex F (informative) Considerations for calculations of the relative level of detection (RLOD) between laboratories as obtained in an interlaboratory study	54
Annex G (informative) Principle of the accuracy profile for validation of quantitative models	57
Annex H (informative) Application of the accuracy profile in the method comparison study	59
Annex I (informative) Example of the application of the accuracy profile for an interlaboratory study	62
Bibliography	66

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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary Information](#)

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition of ISO 16140-2, together with ISO 16140-1, cancels and replaces ISO 16140:2003, which has been technically revised. It also incorporates the Amendment ISO 16140:2003:Amd.1:2011.

ISO 16140 consists of the following parts, under the general title *Microbiology of the food chain — Method validation*:

- *Part 1: Vocabulary*
- *Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

The following parts are under preparation:

- *Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory*
- *Part 4: Protocol for single-laboratory (in-house) method validation*
- *Part 5: Protocol for factorial interlaboratory validation of non-proprietary methods*
- *Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing*

Introduction

Today, many alternative, mostly proprietary, methods exist that are used to assess the microbiological quality of raw materials and finished products and the microbiological status of manufacturing procedures. These methods are often faster and easier to perform than the corresponding standardized method. The developers, end users, and authorities need a reliable common protocol for the validation of such alternative methods. The data generated will also provide potential end users with performance data for a given method, thus, enabling them to make an informed choice on the adoption of a particular method. The data generated can also be the basis for the certification of a method by an independent organization.

This part of ISO 16140

- is intended to provide a specific protocol and guidelines for the validation of proprietary methods intended to be used as a rapid and/or easier method to perform than the corresponding reference method,
- can also be used for the validation of other non-proprietary methods that are used instead of the reference method,
- is intended as the successor of the validation protocol published in the first version of ISO 16140 (ISO 16140:2003), and
- is mainly written for the validation of methods that are capable of culturing the target microorganism, but can also be applied to methods for microorganisms that cannot be cultured such as viruses (e.g. Norovirus) and protozoan parasites (e.g. *Cryptosporidium* or *Giardia*). In these cases, some wordings are to be interpreted so as to fit the situation for non-culturable organisms.

The use of this part of ISO 16140 involves expertise on relevant areas such as microbiology, statistical design, and analysis as indicated in the respective sections. The statistical expertise encompasses overview of sampling theory and design of experiments, statistical analysis of (qualitative and quantitative) microbiological data, and overview of statistical concepts on random sampling, sample heterogeneity, sample stability, design of experiments, and variance components.

When this part of ISO 16140 is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviation from them in the case of particular products.

The harmonization of validation methods cannot be immediate and for certain groups of products, International Standards and/or national standards may already exist that do not comply with this part of ISO 16140. It is hoped that when such standards are reviewed, they will be changed to comply with ISO 16140 so that eventually, the only remaining departures from this part of ISO 16140 will be those necessary for well-established technical reasons. For example, ISO 16297[3] deals with a very specific validation for a specific subject (the hygienic status of raw milk samples) and will remain as a vertical standard besides ISO 16140. If such a validation is needed, the vertical standard is more important.

Microbiology of the food chain — Method validation —

Part 2:

Protocol for the validation of alternative (proprietary) methods against a reference method

1 Scope

This part of ISO 16140 specifies the general principle and the technical protocol for the validation of alternative, mostly proprietary, methods for microbiology in the food chain. Validation studies according to this part of ISO 16140 are intended to be performed by organizations involved in method validation.

This part of ISO 16140 is applicable to the validation of methods for the analysis (detection or quantification) of microorganisms in

- products intended for human consumption,
- products intended for animal feeding,
- environmental samples in the area of food and feed production, handling, and
- samples from the primary production stage.

This part of ISO 16140 is in particular applicable to bacteria and fungi. Some clauses of this part of ISO 16140 could be applicable to other (micro) organisms or their metabolites on a case-by-case-basis. In the future, guidance for other organisms (e.g. viruses and parasites) will be included in either this part or a separate part of ISO 16140.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 16140-1, *Microbiology of the food chain— Method validation — Part 1: Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16140-1 apply.

4 General principles for the validation of alternative methods

The validation protocol comprises two phases:

- a method comparison study of the alternative (proprietary) method against the reference method carried out in the organizing laboratory;
- an interlaboratory study of the alternative (proprietary) method against the reference method carried out in different laboratories.

The technical rules for performing the method comparison study and the interlaboratory study are given in [Clause 5](#) and [Clause 6](#), depending upon whether the alternative (proprietary) method is