SLOVENSKI STANDARD
SIST EN ISO 16140-2:2016

01-oktober-2016

Nadomešča:
SIST EN ISO 16140:2003
SIST EN ISO 16140:2003/A1:2012

Mikrobiologija v prehranski verigi - Validacija metode - 2. del: Protokol za validacijo alternativnih (lastniških) metod glede na referenčno metodo (ISO 16140-2:2016)

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)

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

Ta slovenski standard je istoveten z: **EN ISO 16140-2:2016**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

SIST EN ISO 16140-2:2016 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 16140-2

July 2016

ICS 07.100.30

Supersedes EN ISO 16140:2003

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.

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, 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 United Kingdom.

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

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

Ref. No. EN ISO 16140-2:2016 E

Contents

Page

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.

INTERNATIONAL STANDARD

ISO 16140-2

First edition
2016-06-15

Microbiology of the food chain — Method validation —

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

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

Reference number
ISO 16140-2:2016(E)

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

Contents

Page

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 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 protozan 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

qualitative or quantitative in nature. The data generated in some parts of the validation study are evaluated using the so-called Acceptability Limits (AL) and no statistical evaluation of the data are conducted. These AL are based on experts' opinion and data generated in existing validation studies.

5 Qualitative methods — Technical protocol for validation

5.1 Method comparison study

5.1.1 General considerations

The method comparison study is the part of the validation process that is performed in the organizing laboratory. It consists of three parts namely the following:

— a comparative study of the results of the reference method to the results of the alternative method in (naturally and/or artificially) contaminated samples (so-called sensitivity study);

— a comparative study to determine the relative level of detection (RLOD) in artificially contaminated samples (so-called RLOD study);

— an inclusivity/exclusivity study of the alternative method.

The results (tables and calculations) of the different parts and the interpretation of the results, including discrepant results, shall be given in a study report.

Test portions size shall be used as written in the reference method.

5.1.2 Paired or unpaired study

The reference and alternative methods shall be performed with, as far as possible, exactly the same sample (same test portion). However, a distinction is made between studies where the same test portion can be used for both the reference and the alternative method due to both methods having exactly the same first step in the (enrichment) procedure and those where different test portions need to be used for the reference and the alternative method (e.g. due to different enrichment broths). In the case where the same test portion is used for both methods, the results from both methods are highly related to each other. For example, when the sample is not contaminated, both methods should find the result of that sample negative. Due to this relationship, the data produced by the reference and the alternative method are named **paired** or matched. In this part of ISO 16140, the wording "paired study" will be used for this type of study.

The opposite situation where there is no shared initial (enrichment) step for both the reference and the alternative method is also possible. In this case, different test portions coming from the same batch or lot of product have to be used for the two methods and the resulting data are named **unpaired** or unmatched. In this part of ISO 16140, the word "unpaired study" will be used for this type of study. The choice of having a **paired** study or an **unpaired** study depends on the protocols of the reference and alternative method. If there is a common initial step in the (enrichment) procedures, a **paired** study design is mandatory.

This clause describes the method comparison study if the reference and alternative method have a joint initial step in the (enrichment) procedures (**paired** study) and if the reference and alternative method do not have a joint initial (enrichment) step (**unpaired** study). Differences between both types of studies are indicated in the text where appropriate.

5.1.3 Sensitivity study

The sensitivity study aims to determine the difference in sensitivity between the reference and the alternative method. This study is conducted using naturally and/or artificially contaminated samples. Different categories and types shall be tested for this. Acceptability Limits have been defined for the

maximum acceptable difference depending on the type of study (**paired/unpaired**) and the number of categories tested.

5.1.3.1 Selection of categories to be used

The selection of categories and types used within the validation will depend on the type or group of microorganism and the scope of the validation.

If the method is to be applied for a broad range of foods, then at least five categories of food shall be studied. The validation study report shall state the food categories used in the study. If the method is to be validated for a restricted number of food categories, e.g. "ready-to-eat, ready-to-reheat meat products", and "heat-processed milk and dairy products", then only these categories need to be studied. In addition to food, feed samples, environmental samples, and primary production stage samples can be included as additional categories. This will broaden the application of the use of the alternative method for these additional categories.

For all selected categories (food and others), at least three different types per category shall be included in the study. Annex A presents an overview of the relevant types and categories for specific microorganisms that might be relevant for the validation. Annex A should be used to facilitate the selection of categories, types, and items for the specific microorganism involved. It should not be regarded as a mandatory choice.

When selecting samples for the study, it is of the highest priority to find those that are naturally contaminated. If it is not possible to acquire a sufficient number of naturally contaminated samples, artificial contamination of samples is permissible (see Annex B and Annex C). Details on the preparation of the artificially inoculated samples should be given in the validation study report. It is desirable that food samples come from as wide a distribution as possible in order to reduce any bias from local food specialities and to broaden the range of validation.

It shall be ensured that with the selection of the different types, both high and low (natural) background microflora, different types of stresses due to processing, and raw (unprocessed) items are included in the study.

EXAMPLE For the validation of a method for detection of *Listeria monocytogenes* and the category "ready-to-eat, ready-to-reheat meat products", the types can be (1) cooked meat products (lower background flora, heat stress), (2) fermented or dried meat products (high background flora, pH stress), and (3) raw cured (smoked) (a_w <0,92) (intermediate background flora, a_w stress).

In some cases, for example, for an alternative method that is applicable for a broad range of foods, it is possible to combine the "ready-to-eat" and "raw" categories from the same product group. For example, the categories raw and ready-to-eat meat (products) can be combined into one category having three types divided over relevant raw and ready-to-eat food types. The selection of (combined) food categories should be based on risk analysis.

5.1.3.2 Number of samples

For each category being examined, a minimum of 60 individual samples shall be tested made up of at least three types with at least 20 samples representative for each type (three types × 20 samples for each type = 60 samples). Fractional positive results by either the reference or alternative method (i.e. samples should not be all positive or all negative) shall be obtained for each type tested. In the ideal situation, 10 samples (50 %) tested per type should be positive and 10 negative, but should range between 25 % and 75 %. For each category, at least 30 samples shall have a positive result by the reference and/or the alternative method.

5.1.3.3 Alternative-method result and confirmation

Many alternative-method protocols contain two steps, the first being the enrichment and detection step and the second being the confirmation of the detection result from step one. The end result of the alternative method is the result after step two. The end result will be the same as the result

