Microbiology of the food chain - Method validation - Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory (ISO 16140-3:2021)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 16140-3:2021 sisaldab Euroopa standardi EN ISO 16140-3:2021 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 16140-3:2021 consists of the English text of the European standard EN ISO 16140-3:2021.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation and Accreditation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 03.02.2021.	Date of Availability of the European standard is 03.02.2021.
Standard on kättesaadav Eesti Standardimis-ja Akrediteerimiskeskusest.	The standard is available from the Estonian Centre for Standardisation and Accreditation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 07.100.30

EUROPEAN STANDARD

NORME EUROPÉENNE

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EN ISO 16140-3

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English Version

Microbiology of the food chain - Method validation - Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory (ISO 16140-3:2021)

Microbiologie de la chaîne alimentaire - Validation des méthodes - Partie 3: Protocole pour la vérification dans un seul laboratoire de méthodes de référence et de méthodes alternatives validées (ISO 16140-3:2021)

Mikrobiologie der Lebensmittelkette - Verfahrensvalidierung - Teil 3: Arbeitsvorschrift für die Verifizierung von Referenz- und alternativen Verfahren in einem einzelnen Labor (ISO 16140-3:2021)

This European Standard was approved by CEN on 28 December 2020.

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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.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. EN ISO 16140-3:2021 E

European foreword

This document (EN ISO 16140-3:2021) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 463 “Microbiology of the food chain” the secretariat of which is held by AFNOR.

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 August 2021, and conflicting national standards shall be withdrawn at the latest by August 2021.

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.

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 16140-3:2021 has been approved by CEN as EN ISO 16140-3:2021 without any modification.

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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 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 Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 16140 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.

Introduction

0.1 The ISO 16140 series

The ISO 16140 series has been expanded in response to the need for various ways to validate or verify test methods. It is the successor to ISO 16140:2003. The ISO 16140 series consists of six parts with 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;*

— *Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory;*

— *Part 4: Protocol for method validation in a single laboratory;*

— *Part 5: Protocol for factorial interlaboratory validation for non-proprietary methods;*

— *Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.*

ISO 17468 is a closely linked International Standard, which establishes technical rules for the development and validation of standardized methods.

In general, two stages are needed before a method can be used in a laboratory.

— The first stage is the validation of the method. Validation is conducted using a study in a single laboratory followed by an interlaboratory study (see ISO 16140-2, ISO 16140-5 and ISO 16140-6). In the case when a method is validated within one laboratory (see ISO 16140-4), no interlaboratory study is conducted.

— The second stage is method verification, where a laboratory demonstrates that it can satisfactorily perform a validated method. This is described in this document (i.e. ISO 16140-3). Verification is only applicable to methods that have been validated using an interlaboratory study.

In general, two types of methods are distinguished: reference methods and alternative methods.

A reference method is defined in ISO 16140-1:2016, 2.59, as an "internationally recognized and widely accepted method". The note to entry clarifies that "these are ISO standards and standards jointly published by ISO and CEN or other regional/national standards of equivalent standing".

In the ISO 16140 series, reference methods include standardized reference (ISO and CEN) methods as defined in ISO 17468:2016, 3.5, as a "reference method described in a standard".

An alternative method (method submitted for validation) is defined in ISO 16140-1:2016, 2.4, as a "method of analysis that detects or quantifies, for a given category of products, the same analyte as is detected or quantified using the corresponding reference method". The note to entry clarifies that: "The method can be proprietary. The term 'alternative' is used to refer to the entire 'test procedure and reaction system'. This term includes all ingredients, whether material or otherwise, required for implementing the method".

ISO 16140-4 addresses validation within a single laboratory. The results are therefore only valid for the laboratory that conducted the study. In this case, verification (as described in this document) is not applicable. ISO 16140-5 describes protocols for non-proprietary methods where a more rapid validation is required or when the method to be validated is highly specialized and the number of participating laboratories required by ISO 16140-2 cannot be reached. ISO 16140-4 and ISO 16140-5 can be used for validation against a reference method. ISO 16140-4 (qualitative and quantitative) and ISO 16140-5 (quantitative only) can also be used for validation without a reference method.

The flow chart in Figure 1 gives an overview of the links between the different parts mentioned above. It also guides the user in selecting the right part of the ISO 16140 series, taking into account the purpose of the study and the remarks given above.

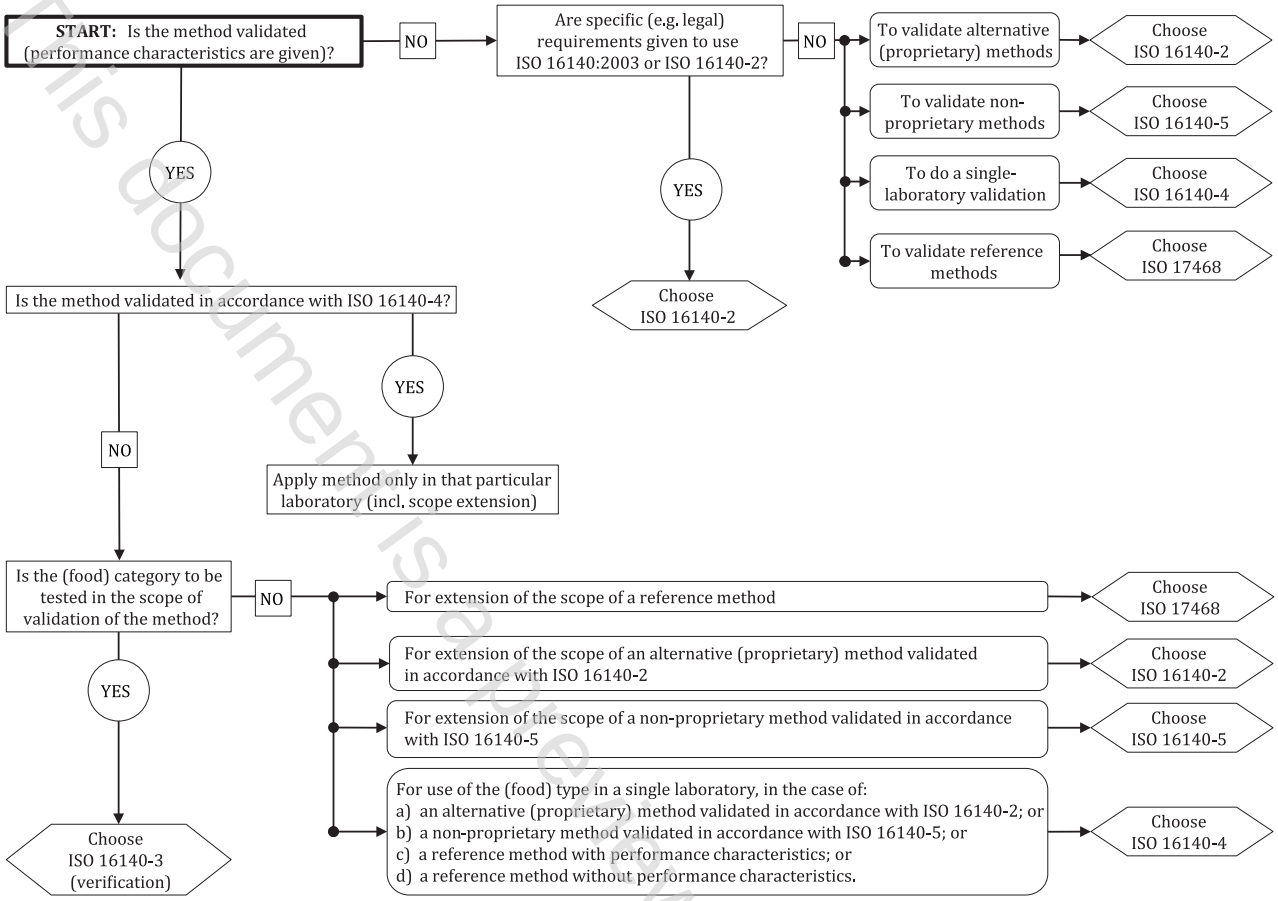

Figure 1 — Flow chart for application of the ISO 16140 series

NOTE 1 In this document, the words “category”, “type” and/or “item” are sometimes combined with “(food)” to improve readability. However, the word “(food)” is interchangeable with “(feed)” and other areas of the food chain as mentioned in Clause 1.

NOTE 2 The general principle for method verification is that the method to be verified (either alternative or reference) has been validated. However, some reference methods (including ISO or CEN standards) are not yet (fully) validated. For verification of these methods, the protocols are described in Annex F.

ISO 16140-6 is somewhat different from the other parts in the ISO 16140 series in that it relates to a very specific situation where only the confirmation procedure of a method is to be validated [e.g. the biochemical confirmation of *Enterobacteriaceae* (see ISO 21528-2)]. The confirmation procedure advances a suspected (presumptive) result to a confirmed positive result. The validation of alternative typing techniques (e.g. serotyping of *Salmonella*) is also covered by ISO 16140-6. The validation study in ISO 16140-6 clearly defines the selective agar(s) from which strains can be confirmed using the alternative confirmation method. If successfully validated, the alternative confirmation method can only be used if strains are recovered on an agar that was used and shown to be acceptable within the validation study. Figure 2 shows the possibilities where an alternative confirmation method validated in accordance with ISO 16140-6 can be applied (see text in the boxes).

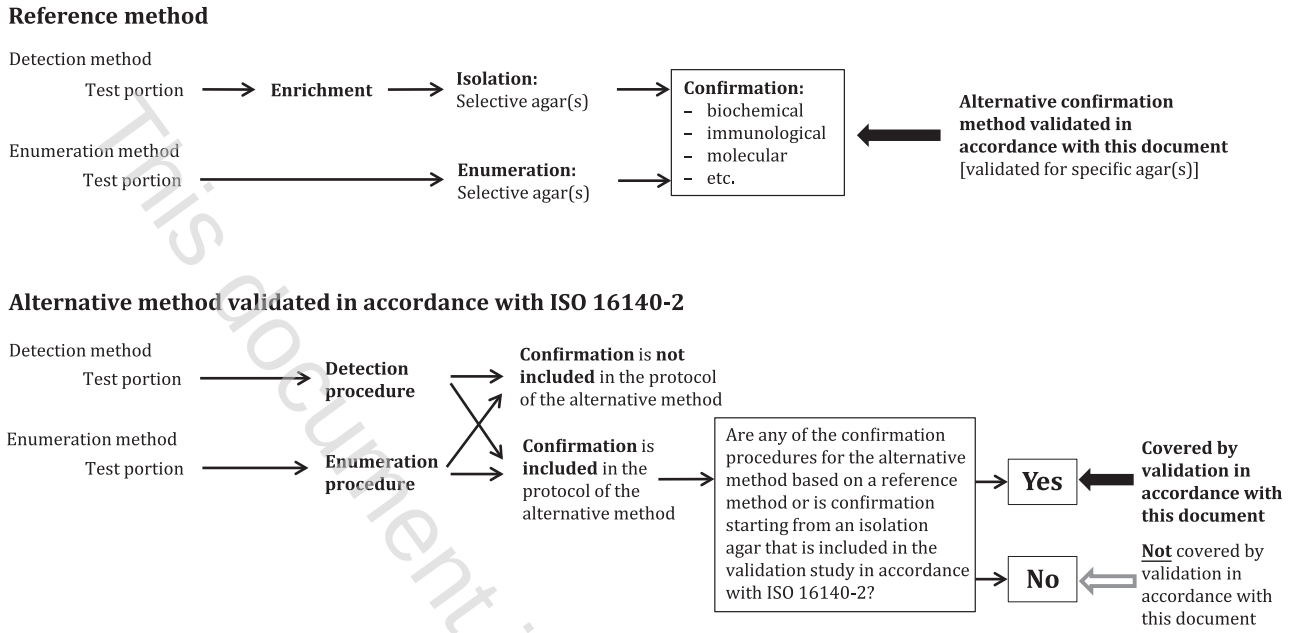

Figure 2 — Use of validated alternative confirmation methods (see ISO 16140-6)

EXAMPLE An example application of a validated alternative confirmation method is as follows.

An alternative confirmation method based on ELISA has been validated to replace the biochemical confirmation for *Salmonella* as described in ISO 6579-1. In the validation study, XLD (mandatory agar in accordance with ISO 6579-1) plus BGA and a specified chromogenic agar (two optional agars for second plating in accordance with ISO 6579-1) were used as the agars to start the confirmation. The validated confirmation method can be used to replace the biochemical confirmation under the following conditions:

— by laboratories using the ISO 6579-1; or

— by laboratories using an ISO 16140-2 validated alternative method that refers to ISO 6579-1 for confirmation; or

— by laboratories using an ISO 16140-2 validated alternative method that starts the confirmation from XLD and/or BGA agar and/or the specified chromogenic agar.

The validated confirmation method cannot be used under the following conditions:

— by laboratories using an ISO 16140-2 validated alternative method that refers only to agars other than those included in the validation to start the confirmation (e.g. Hektoen agar and SS agar only); or

— by laboratories using an ISO 16140-2 validated alternative method that refers only to a confirmation procedure that does not require isolation on agar.

0.2 Verification versus validation

ISO 16140-1:2016 defines the terms for validation and verification, as follows:

— **validation:** establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled;

— **verification:** demonstration that a validated method performs, in the user's hands, according to the method's specifications determined in the validation study and is fit for its intended purpose.

NOTE 1 The user's hand means the user laboratory.

Method verification applies to methods that are:

— reference methods, including ISO or CEN standards, that are validated using at least an interlaboratory study;

NOTE 2 However, some reference methods (including ISO or CEN standards) are not yet (fully) validated. For verification of these methods, the protocols are described in Annex F.

— alternative methods, proprietary or otherwise, when the validation included an interlaboratory study. The method has been validated in accordance with

  — ISO 16140-2 for alternative (proprietary) methods,

  — ISO 16140-5 for non-proprietary methods, or

  — ISO 16140-6 for alternative (proprietary) confirmation and typing methods.

In a validation study, it is not possible to test all existing foods; the diversity and number of samples used in any validation study is limited. In most cases, the validation is based on five different food categories (categories as defined in ISO 16140-1:2016, 2.11, and specified in ISO 16140-2:2016, Annex A). Sometimes the validation is supplemented with additional (other) categories such as pet food and animal feed, environmental samples (food or feed production), and/or primary production samples.

When a minimum of five different food categories are validated, the method is regarded as being validated for a "broad range of foods". And even though only five food categories are tested during the validation study, the method is expected to work for any type of food samples within the 15 food categories in ISO 16140-2: 2016, Annex A. In other words, the "scope" of validation of the method is a broad range of foods, corresponding to the 15 food categories included in ISO 16140-2:2016, Annex A. The scope of validation is important for selecting categories, types and items for the verification.

Two kinds of verification are distinguished:

— The first one is named **implementation verification**. Its purpose is to demonstrate that the user laboratory is able to perform the method correctly. The user laboratory tests a (food) item that was used in the validation study (for qualitative methods) and any (food) item within the scope of validation (for quantitative methods) and then compares the result obtained from the verification to the result obtained from the validation.

— The second one is named **(food) item verification**. Its purpose is to demonstrate that the user laboratory is capable of testing the (food) items it claims in the scope of laboratory application. The user laboratory tests (food) items included in the scope of validation that are commonly examined by the user. As not all (food) items can be included in the verification, the user laboratory is asked to test challenging (food) items.

The scope specifies the (group of) products – categories or types or items – for which the method can be applied. Different scopes are distinguished:

— **scope of the method:** (group of) products – categories or types or items – for which the method is claimed to be applicable.

— **scope of validation:** (group of) products – categories or types or items – for which the applicability of the method is claimed to be validated.

  NOTE The claim for the scope of validation is in most cases wider than the products that are included in the validation study itself. For example, in the case of alternative (proprietary) methods validated in accordance with ISO 16140-2:2016: if at least five (≥ 5) food categories – by using a minimum of three different food types per category – were tested in the validation study, then the scope of validation is a "broad range of foods" (so all 15 food categories are claimed in the scope of validation). When less than five (< 5) food categories were tested, the scope of validation is limited to only those food categories included in the validation.

— **scope of laboratory application**: (group of) products – categories or types or items – for which the method is claimed to be used by the laboratory and are within the scope of validation.

The overlap between the different scopes (including an example) is illustrated in Figure 3.

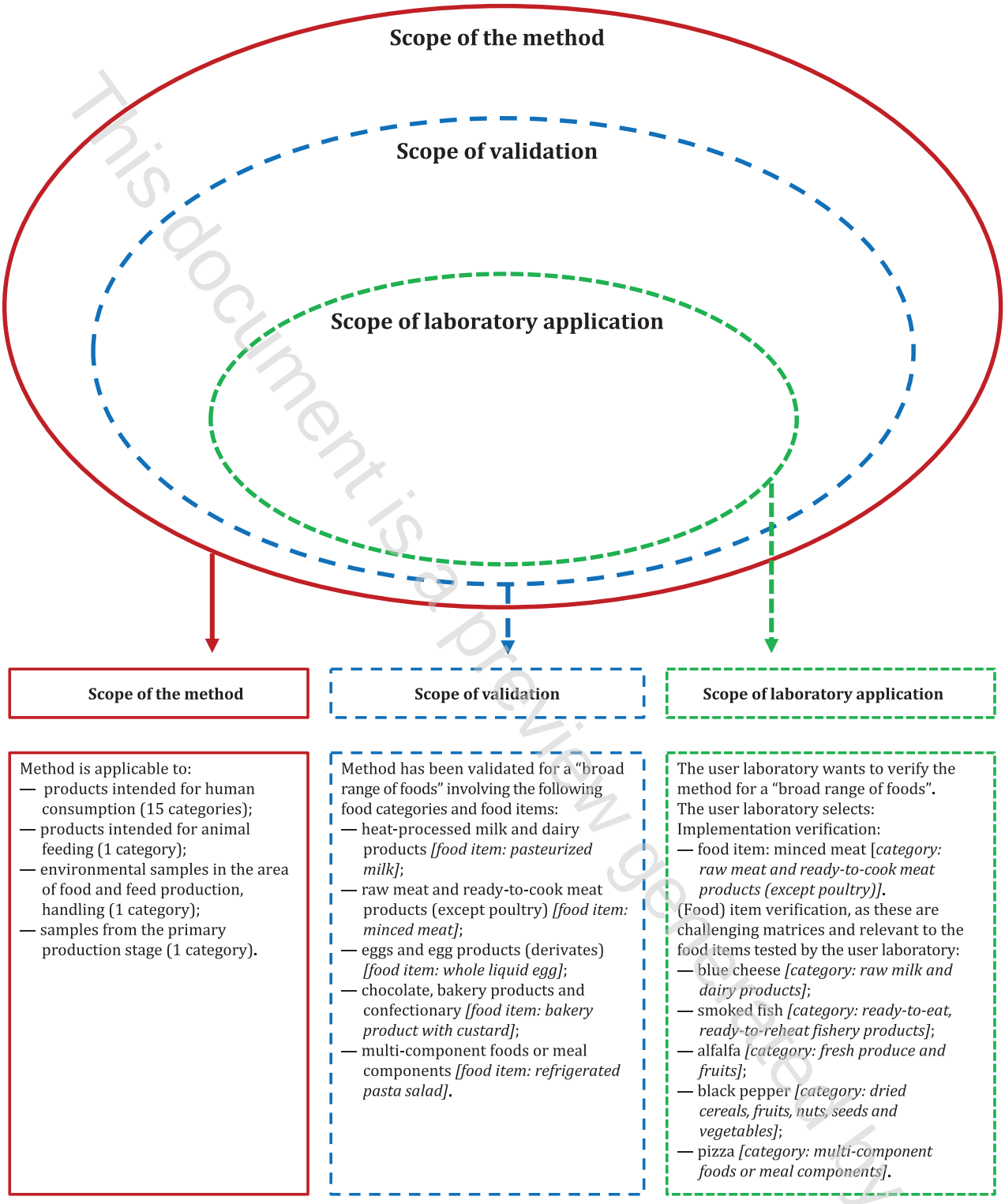

Figure 3 — Overlap between the different scopes (including an example)

At the time of publication of this document (i.e. ISO 16140-3:2021), some reference methods are not yet (fully) validated and would therefore fall outside the scope of this document. It is recognized that standardization organizations (including ISO and CEN committees) will need time to validate their reference methods. Therefore, these non-validated reference methods (including ISO or CEN standards) are verified in a user laboratory according to a specific protocol (see Annex F). This is seen as a temporary situation until these methods are validated by the ISO and/or CEN committees. For further information, see Reference [13].

In this document:

— “shall” indicates a requirement;

— “should” indicates a recommendation;

— “may” indicates a permission;

— “can” indicates a possibility or a capability.

Information marked “NOTE” is for guidance in understanding or clarifying the associated sentence.

Microbiology of the food chain — Method validation —

Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory

1 Scope

This document specifies the protocol for the verification of reference methods and validated alternative methods for implementation in the user laboratory.

This document is applicable to the verification of methods used for the analysis (detection and/or quantification), confirmation and typing of microorganisms in:

— products intended for human consumption;

— products intended for animal feeding;

— environmental samples in the area of food and feed production, handling;

— samples from the primary production stage.

This document is, in particular, applicable to bacteria and fungi. Some clauses can be applicable to other (micro)organisms or their metabolites, to be determined on a case-by-case basis.

The technical protocols for the verification of validated qualitative methods and validated quantitative methods are described in Clauses 5 and 6. The technical protocol for the verification of validated alternative confirmation and typing methods is described in Clause 7. The protocols for the verification of non-validated reference methods are described in Annex F.

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 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 16140-1:2016, *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 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/