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Microbiology of the food chain — Method validation —

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

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

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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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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.

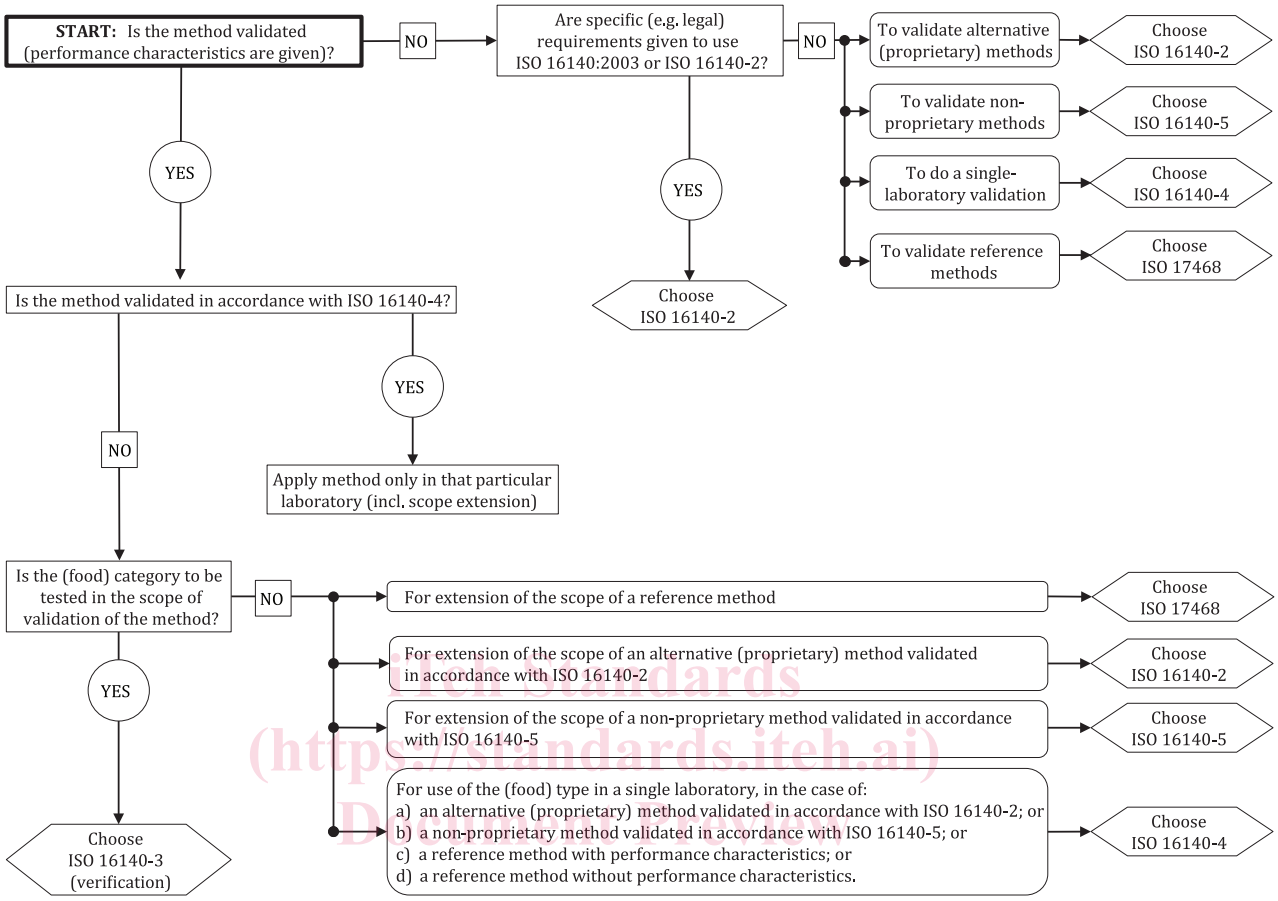

Figure 1 — Flow chart for application of the ISO 16140 series

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).