FINAL DRAFT
Amendment

ISO 16140-4:2020/ FDAM 1

Microbiology of the food chain — Method validation —

Part 4: Protocol for method validation in a single laboratory

AMENDMENT 1: Validation of a larger test portion size for qualitative methods

Microbiologie de la chaîne alimentaire — Validation des méthodes —

Partie 4: Protocole pour la validation de méthodes dans un seul laboratoire

AMENDEMENT 1: Validation d'une taille de prise d'essai plus grande pour des méthodes qualitatives

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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).

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Microbiology of the food chain — Method validation —

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AMENDMENT 1: Validation of a larger test portion size for qualitative methods

Clause 3

Add the following terminological entry:

3.13
larger test portion size
measured (volume or mass) representative sample taken from the laboratory sample or test sample for use in the preparation of the initial suspension that is larger than the test portion that has been described in the original method and/or validation document

4.1

Add the following text after the second paragraph:

The protocol used to validate a larger test portion size for qualitative methods in a single laboratory shall be as given in Annex H.

Annex H

Add the following annex after Annex G, before the Bibliography.

Annex H
(normative)

Validation of a larger test portion size for qualitative methods

H.1 General

This annex specifies a protocol for the validation of qualitative methods when using a larger test portion size. This protocol is intended to demonstrate the effect of analysing a test portion larger than the test portion initially used to validate the method. Validation of the larger test portion size applies only to the laboratory conducting the study and only to the specific (food) category used.

Qualitative reference methods which were validated using a larger test portion size in accordance with ISO 17468 and qualitative alternative (proprietary) methods which were validated using a larger test portion size in accordance with ISO 16140-2 only need to be verified by a laboratory following ISO 16140-3.

The larger test portion size can be used in other laboratories once this has been validated in a study in accordance with ISO 16140-2 or ISO 16140-5. See the flow diagram in Figure 1. Once validated in such a study, any laboratory can implement the larger test portion size after verification following ISO 16140-3. For the verification of a larger test portion size, the same (food) category shall be used. If not validated in accordance with ISO 16140-2 or ISO 16140-5, validation in accordance with the protocol in this annex is necessary for each laboratory wishing to use a larger test portion size.

Requirements with regards to pooling are specified in ISO 6887-1.

When testing a larger single test portion, pooled test portion or pooled (pre-)enrichment test portion, the dilution ratio (sample/diluent) used in the validated method shall remain the same as well as the other incubation conditions (e.g. time and temperature). This ratio may be increased to overcome the inhibitory effects coming from certain food materials as those mentioned in ISO 6887-4:2017, 9.1.4.4 (e.g. onion powder, garlic, oregano, peppers, certain teas and coffees, vitamin premixes, highly salted products).

A reduction in, for example, the dilution ratio (sample/diluent) requires a validation study (details are given in Figure H.1).

Items can either be composited, pooled out of the laboratory or in the laboratory as a test portion or as a (pre-)enriched test portion but not as two or more combinations [i.e. pooling of (pre-)enriched as well as test portion is not allowed].

When a laboratory, for example, wants to pool test portions as well as (pre-)enriched test portions, two separate validation studies shall be conducted in accordance with this protocol.

Once the larger test portion size has been validated, all test portions smaller than the largest validated test portion can be used for routine testing for this particular (food) category at the same sample/diluent ratio. For example, a method that has been validated for 375 g test portions can be used for 25 g, 100 g, etc., up to 375 g test portions.

The protocol is intended to demonstrate that a larger test portion size provides a similar or lower level of detection (LOD_{50}) compared to the LOD_{50} of the (validated) test portion size as specified in the method. The relative level of detection (RLOD) approach specified in 6.1.1.3 shall be used. For calculation of the data, the larger test portion size corresponds to the alternative method and the original test portion size corresponds to the reference method.