Webinar: Publication ISO 16140-3 ‘Method verification’ – improving confidence in laboratory results

Program and speakers

Opening and welcome by hosts Paul in ‘t Veld and Laura Mout

Convenor and Secretary of ISO/TC 34/SC 9/WG 3 ‘Method validation’

Official presentation of ISO 16140-3 ‘Method verification’ by DeAnn Benesh and Benjamin Diep

Project leaders of ISO 16140-3

Questions and Answers (Q&A)

Closing words by Bertrand Lombard or Gwénola Hardouin

Chair and Committee Manager of ISO/TC 34/SC 9 ‘Microbiology’

Introduction

Working Group 3 ‘Method validation’ of ISO/TC 34/SC 9 ‘Food products - Microbiology’ is responsible for the ISO standards on **method validation and verification**

WG 3 ‘Method validation’:

- started in 2006
- with 100 experts coming from 23 countries, a representation of:
  - government
  - industry
  - laboratories
  - academic and research bodies
  - method developers and validation bodies
- developed 7 standards and more standards will follow

ISO 16140 series and ISO 17468

ISO 16140 ‘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 ‘Microbiology of the food chain - Technical requirements and guidance on establishment or revision of a standardized reference method’

Overview of ISO 16140-3:2021

'Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory'


INTERNATIONAL STANDARD

ISO 16140-3

First edition
2021-01

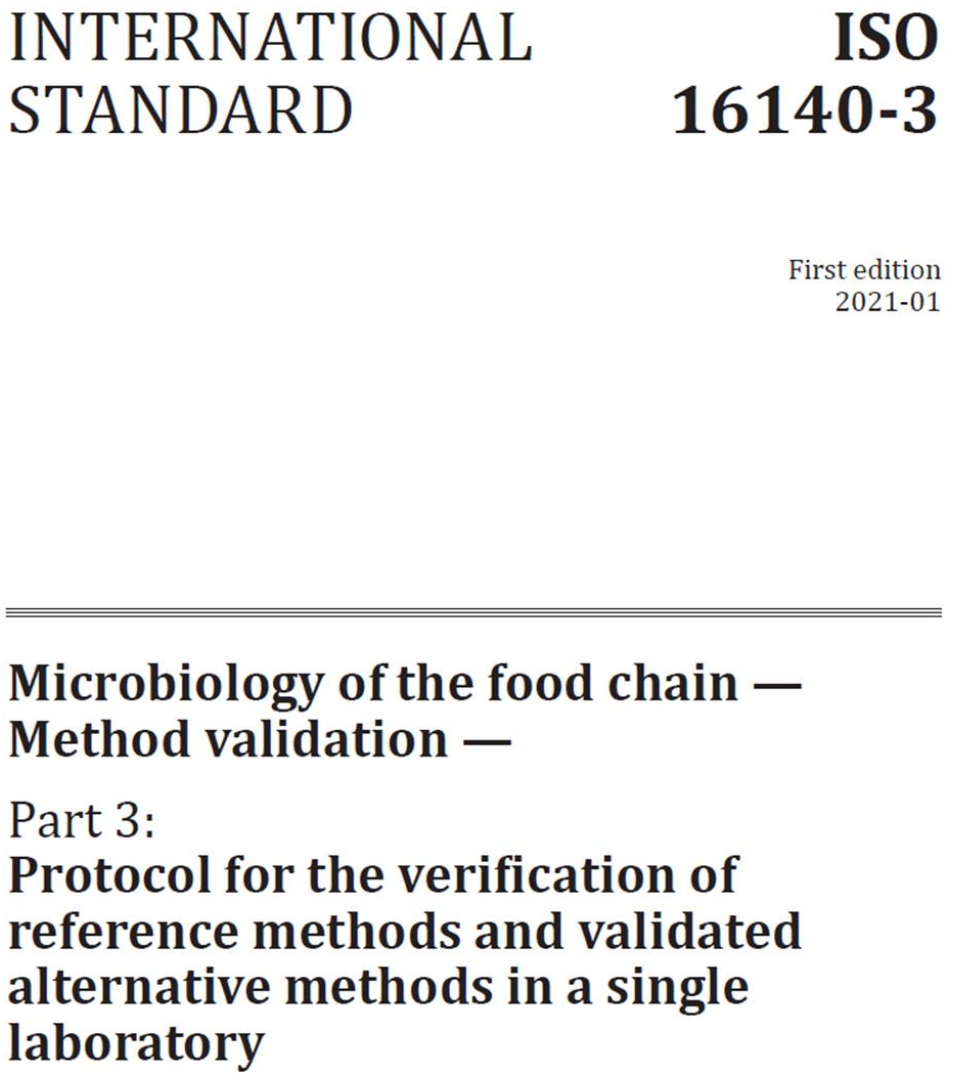
Version: 2 March 2021

Objectives of this presentation

Familiarize you with ISO 16140-3

Help you understand:

- **Why** verification is done
- **What** methods can be verified
- **How** to verify methods
- **When** ISO 16140-3 will be implemented


INTERNATIONAL STANDARD

ISO 16140-3

First edition
2021-01

Microbiology of the food chain — Method validation —

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

Why is this standard on verification needed?

ISO 17025 requirement

> *"The laboratory shall verify that it can properly perform methods before introducing them by ensuring that it can achieve the required performance. Records of the verification shall be retained."*

Not many protocols for verification available

- Differences – agency or country specific

Standard created with international input and consideration

Clauses of ISO 16140-3

- **Introduction** and overview (+ Clauses 1-3)
- **General principles** (Clause 4)
- **Qualitative** methods (Clause 5)
- **Quantitative** methods (Clause 6)
- **Confirmation** and **typing** methods (Clause 7)

Additional information covered

- **Non-validated** methods (Annex F)
- *Transition period for the implementation of ISO 16140-3*

Why do we need to validate and verify methods?

Distinguishing validation and verification

from ISO 16140-1:2016 and ISO 16140-3:2021

2.81 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***

2.83 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

Method validation – Reference methods

ISO 17468:2016 ***'Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method'***

International Journal of Food Microbiology 288 (2019) 1–2

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Editorial

European and International validation of 15 main reference methods in the microbiology of the food chain

https://www.sciencedirect.com/journal/international-journal-of-food-microbiology/vol/288/suppl/C

Definitions from ISO 16140-1:2016

2.59 reference method
internationally recognized and widely accepted method

2.4 alternative method (method submitted for validation)
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* (2.59)

Note 1 to entry: 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.

Method validation – Alternative methods

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'

This document specifies the general principle and the technical protocol for the validation of alternative, **mostly proprietary, methods** for microbiology in the food chain.

ISO 16140-6:2019

'Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures'

This document specifies the general principle and the technical protocol for the validation of **alternative confirmation methods** for microbiology in the food chain.

Certification bodies: using ISO 16140-2 and ISO 16140-6

Method validation certificates and reports on their websites:

- https://microval.org/en/issued-certificates/
- https://nf-validation.afnor.org/en/food-industry/#discover-certified-methods
- https://www.nmkl.org/index.php/en/nordval

What about other validated methods?

“Fully validated” method:

- **Comparative study** – method compared to a reference method
- **Interlaboratory study** – method used with same (food) items in many laboratories

Interlaboratory study (ILS):

- ISO 16140-2
- ISO 17468
- AOAC INTERNATIONAL
  ~~***AOAC® Performance Tested Methods℠*** **(PTM)**~~
  AOAC® Official Method of Analysis℠ **(OMA)**

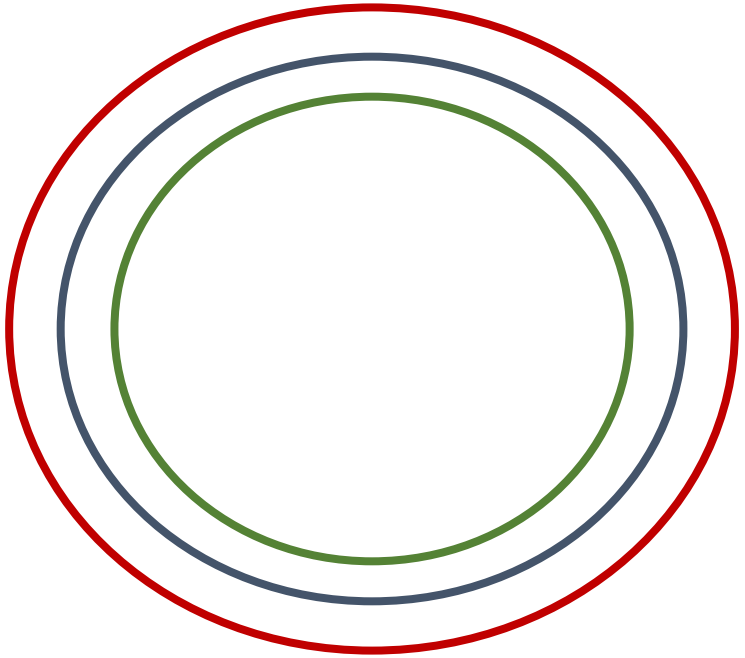
Verification: two stages

1. **Implementation verification**
   - Demonstrate the user laboratory can ***run the method correctly***
   - Verify using ONE (food) item

2. **(Food) item verification**
   - Demonstrate the user laboratory can run the method with the ***(food) items claimed by the user laboratory*** *(laboratory application)*
   - Verify using categories tested in your laboratory

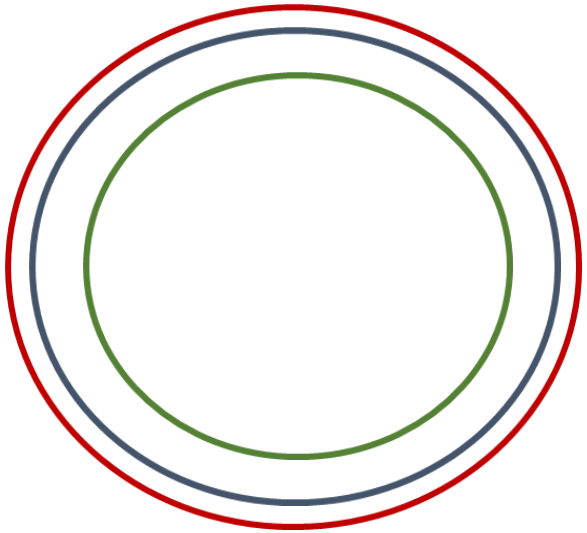
Scope of Method *vs* Validation *vs* Laboratory application

Method	Validation	Laboratory
It specifies the (group of) products (categories or types or items) for which the method is claimed to be *applicable*	It specifies the (group of) products (categories or types or items) for which the method is claimed to be *validated*	It specifies the (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

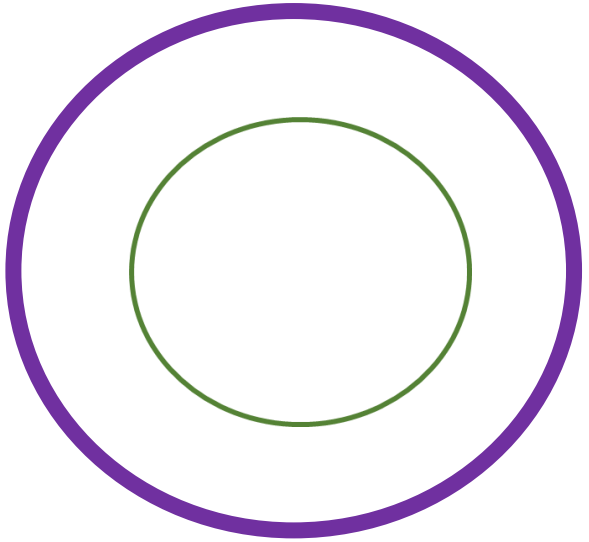


Overlap of Different Scopes - EXAMPLES

Method Scope
– Broad Range of Foods + 3 others
Validation Scope
– Broad Range of Foods + 1 other
Laboratory Application
– Broad Range of Foods



Method Scope
– Broad Range of Foods + 3 other
Validation Scope
– Broad Range of Foods + 3 other
Laboratory Application
– Limited Range of Foods



ISO 16140-3: Scope *[Clause 1]* in relation to Annex A

Classification of (food) categories and suggested target combinations for ***verification*** studies

Categories					
Raw milk and dairy products	Heat-processed milk and dairy products	Raw meat and ready-to-cook meat products (except poultry)	Ready-to-eat, ready-to-reheat meat products	Raw poultry and ready-to-cook poultry products	Ready-to-eat, ready-to-reheat meat poultry products
Eggs and egg products (derivatives)	Raw and ready-to-cook fish and seafoods (unprocessed)	Ready-to-eat, ready-to-reheat fishery products	Fresh produce and fruits	Processed fruits and vegetables	Dried cereals, fruits, nuts, seeds and vegetables
Infant formula and infant cereals	Chocolate, bakery products and confectionary	Multi-component foods or meal components	Pet food and animal feed	Environmental samples (food or feed production)	Primary production samples (PPS)

Same categories are provided in ISO 16140-2:2016, Table A.1, for ***validation studies.*

Normative references *[Clause 2]*

ISO 6887 (all parts) *'Preparation of test samples, initial suspension and decimal dilutions for microbiological examination'*

ISO 7218 *'General requirements and guidance for microbiological examinations'*

ISO 16140-1 *'Method validation - Part 1: Vocabulary'*

Terms and definitions *[Clause 3]*

A total of 21 terms and definitions - 4 are unique to this standard:

- estimated bias
- estimated LOD_{50}
- scope of laboratory application
- user laboratory

General principles *[Clause 4]*

Implementation verification

Demonstrate competence of the user laboratory to perform the method

- **Qualitative** methods:
  - select 1 (food) item from the validation study also within the **scope of laboratory application**
  - use this **1 (food) item and the sample size** used in the validation study to perform implementation verification
- **Quantitative** methods:
  - select any (food) item within the scope of validation of the method

5 food categories tested *(= broad range of foods)* + 2 other categories

Table A.1: Classification of categories and suggested target combinations for *verification* studies

Raw milk and dairy products	Heat-processed milk and dairy products	Raw meat and ready-to-cook meat products (except poultry)	Ready-to-eat, ready-to-reheat meat products	Raw poultry and ready-to-cook poultry products	Ready-to-eat, ready-to-reheat meat poultry products
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Infant formula and infant cereals	Chocolate, bakery products and confectionary	Multi-component foods or meal components	Pet food and animal feed	Environmental samples (food or feed production)	Primary production samples (PPS)

***Implementation* verification:**

- **Qualitative**: powdered egg
- **Quantitative**: pasteurized milk

Presented by ISO/TC 34/SC 9/WG 3 'Method validation'

(Food) item verification

Demonstrate the competence of the user laboratory to perform the validated method with (food) items that are tested in the user laboratory

The user laboratory shall:

1. select **1 challenging (food) item from each (food) category listed within the scope of validation**, that is also a (food) category that is tested within the **scope of laboratory application** of the user laboratory, and

2. use this **1 (food) item** to perform the (food) item verification

Scope: limited range of foods

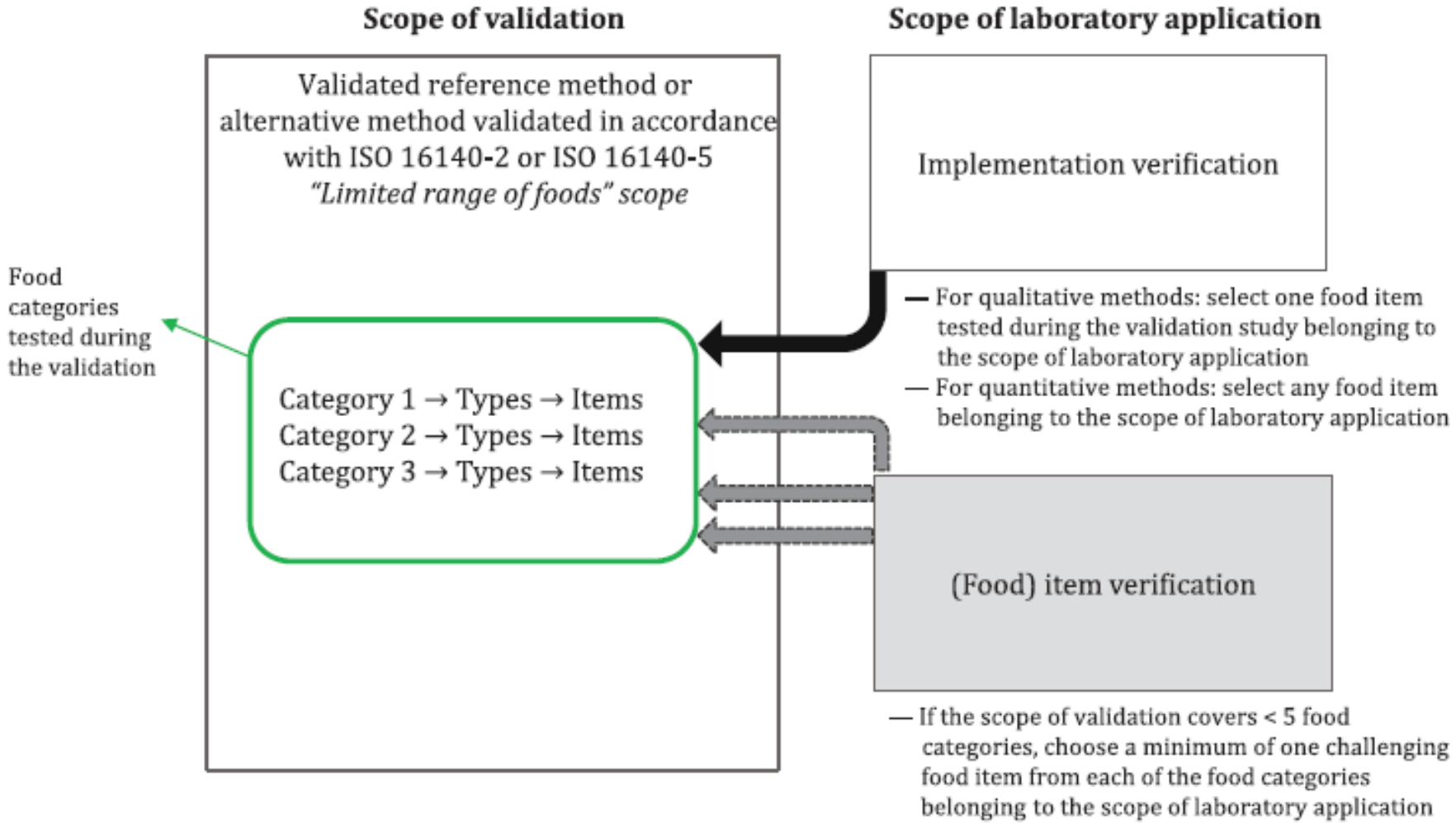


Figure 5 — Food items required when verifying a method for a "limited range of foods" scope

Scope: broad range of foods and other categories

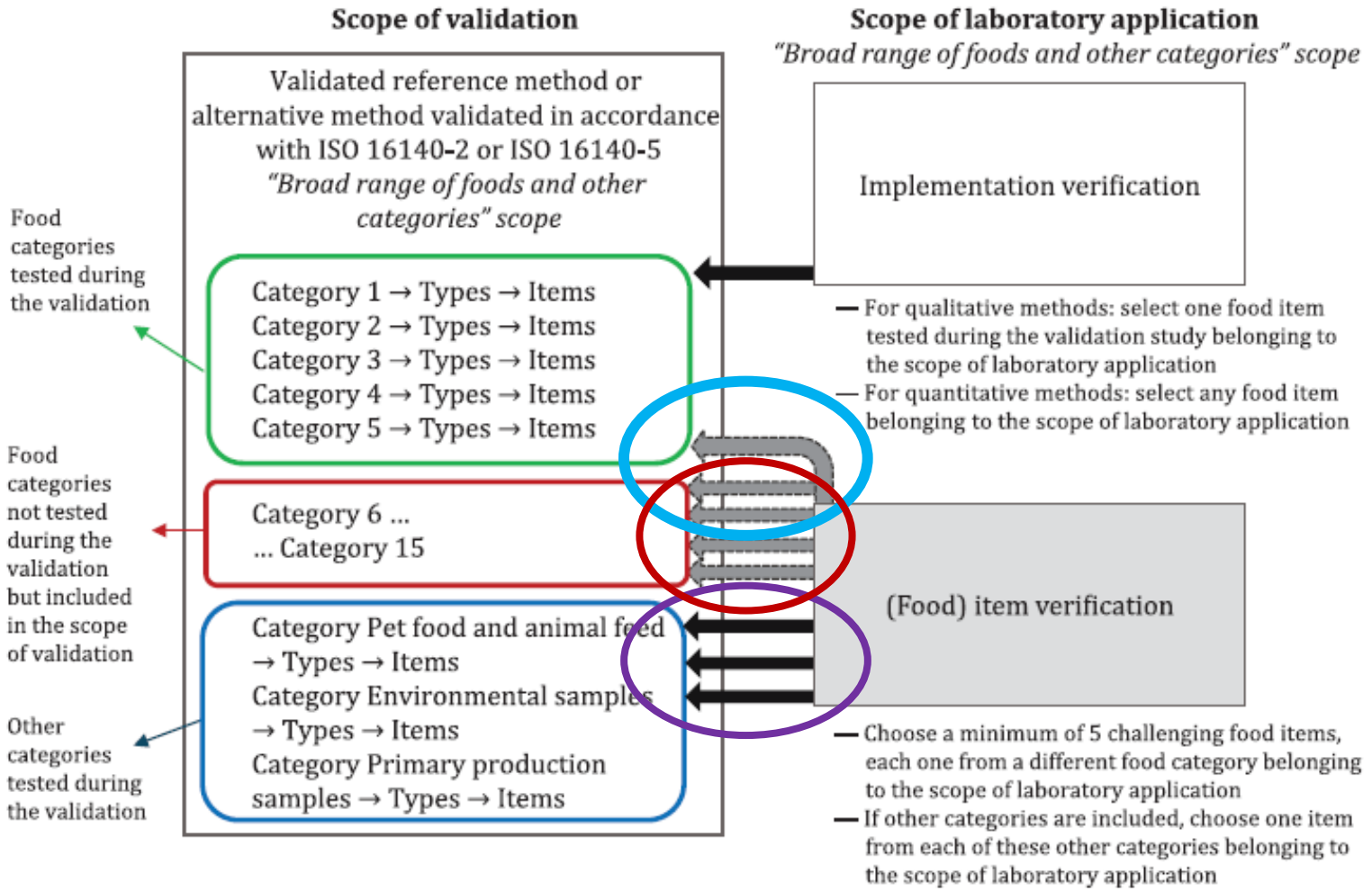


Figure 6 — Items required when verifying a method for a "broad range of foods and other categories" scope

Number of (food) items to test

Table 1 — Summary of the minimum number of (food) items required for verification

Scope of validation	Number of samples		
	Implementation verification	(Food) item verification	Total
"Broad range of foods" scope ≥ 5 food categories	1	≥ 5	≥ 6
"Limited range of foods" scope N_{food} categories	1	$N_{food} \leq 4$	$(N_{food} + 1) \leq 5$
"Broad range of foods" + other categories (N_{other}) scope	1	≥ 5 food items + 1 item from each of the N_{other} other categories	≥ 6 + N_{other}
"Limited range of foods" N_{food} categories + other categories (N_{other}) scope	1	$N_{food} \leq 4$ + 1 item from each of the N_{other} other categories	$(N_{food} + N_{other} + 1) \leq 8$
Other categories (N_{other}) scope only	1	$N_{other} \leq 3$	$(N_{other} + 1) \leq 4$

8

Guidance on how to choose challenging (food) item(s) *[Annex B]*

B.2 Matrix effects to consider:

- **high background microbiota samples**, e. g. poultry minced meat, faecal samples, raw milk
- **spoilage microorganisms:** the presence of this native microbiota can influence the recovery and growth of the target microorganism
- **technological microbiota** such as microbial cultures and probiotics
- **composition**, e. g. high fat content, lecithin, thickener, nutrient content
- **pH**, e. g. pH < 4 to 5 (beverages, sauces, etc.)
- **oxidation reduction potential**
- **water activity**, e. g. a_w < 0,85 (flour, low moisture foods)
- **antimicrobial constituents and growth inhibitors**, e. g. polyphenols, and others

Scope: broad range of foods and other categories

Scope: broad range of foods & other categories

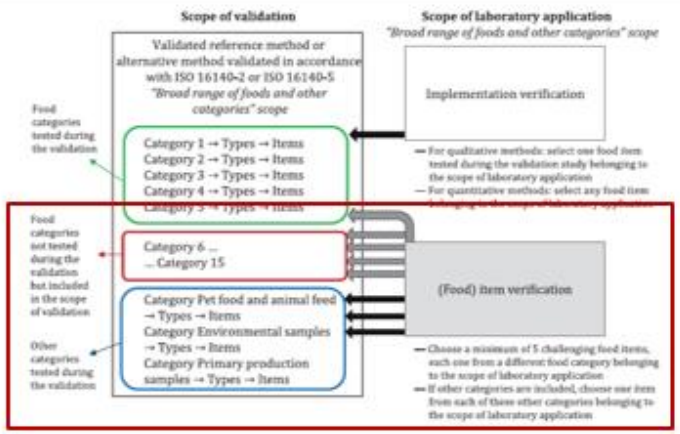


Table A.1: Classification of categories and suggested target combinations for verification studies

Raw milk and dairy products	Heat-processed milk and dairy products	Raw meat and ready-to-cook meat products (except poultry)	Ready-to-eat, ready-to-reheat meat products	Raw poultry and ready-to-cook poultry products	Ready-to-eat, ready-to-reheat meat poultry products
Eggs and egg products (derivatives)	Raw and ready-to-cook fish and seafoods (unprocessed)	Ready-to-eat, ready-to-reheat fishery products	Fresh produce and fruits	Processed fruits and vegetables	Dried cereals, fruits, nuts, seeds and vegetables
Infant formula and infant cereals	Chocolate, bakery products and confectionary	Multi-component foods or meal components	Pet food and animal feed	Environmental samples (food or feed production)	Primary production samples (PPS)

Category *(claimed, not tested)*	Item	Characteristic
Raw poultry and ready-to-cook poultry products	Seasoned chicken breast	High background
Processed fruits and vegetables	Pickle	Low pH
Chocolate, bakery products and confectionary	Custard confectionary	High fat content
Heat-processed milk and dairy products	Ice cream	Lecithin
Eggs and egg products	Egg powder	Low a_w
Pet food and animal feed	Dry dog food pellets	Low a_w
Environmental samples (food or feed production)	Swabs	Low a_w

Performance characteristics

Table 2 — Required performance characteristics to be determined for verification

Method	Performance characteristic	Implementation verification	(Food) item verification
Qualitative	Estimated LOD_{50} ($eLOD_{50}$)	✓	✓
Quantitative	Intralaboratory reproducibility standard deviation (S_{IR})	✓	Not applicable
	Estimated bias (eBias)	Not applicable	✓

NOTE 1 The relationship between intralaboratory reproducibility standard deviation (S_{IR}) and ISO 19036 is explained in 6.1.

NOTE 2 For the verification of qualitative method, three protocols are proposed to the user laboratory. The protocol 3 does not require a determination of an $eLOD_{50}$ but to target a concentration of 3 cfu to 5 cfu/test portion.

No stressed cells required

- **$eLOD_{50}$** – Three available protocols to determine the $eLOD_{50}$
- **S_{IR}** – Design is aligned with ISO 19036:2019
- **eBias** – Analyze in parallel the method to be verified with the (food) item versus inoculum for three levels of inoculation

Workflow: outline

1. Choice of the method to be verified
2. Scope of validation of the method
3. Scope of the verification
4. Select (food) items
5. Protocol for verification
6. Analysis
7. Evaluation of results

Qualitative method verification *[Clause 5]*

Implementation and (food) item verification

Qualitative method verification

Estimated LOD_{50} ($eLOD_{50}$) determination required for both:

1. Implementation verification: follow one of the technical protocols outlined
2. (Food) item verification: apply the **same** technical protocol

> **3.5 estimated LOD_{50}**
>
> determination of the LOD_{50} (level of detection at 50 % probability of detection) based on the experimental design described in this document
>
> Note 1 to entry: An accurate determination of the LOD_{50} is not possible as the number of samples tested is small in comparison to the number of samples required in ISO 16140-2:2016. Therefore, the term "estimated LOD_{50}" ("$eLOD_{50}$") is used in this document.

Annex C provides guidance and examples on preparation of samples and test portions

Implementation verification

ISO 6579-1:2017 *'Salmonella'* validation study

Qualitative *method verification*

LOD_{50} for fresh cheese curd sample = 5,7 cfu/test portion

Table C.1 — Results of data analysis obtained with fresh cheese curd samples

Parameter	Fresh cheese curd (blank)	Fresh cheese curd (low level contamination)[a]	Fresh cheese curd (high level contamination)[a]
Number of participating collaborators	23	23	23
Number of samples per collaborator	5	5	5
Number of collaborators retained after evaluation of the data	21	21	21
Number of samples retained after evaluation of the data	105	105	105
Test portion size, in g	25	25	25
Specificity, in %	100	—	—
Sensitivity, in %	—	74,3	83,8
LOD_{50} (95 % confidence interval), in cfu/test portion	—	5,7 (4,0 to 8,1)	

[a] Cheese samples were artificially contaminated with *Salmonella* Montevideo (lactose positive strain).

Most probable number (MPN) results of the artificially contaminated samples were the following:

	MPN/25 g
Low level	0,7 (0,2 to 2,4)
High level	37,2 (7,5 to 95,0)

Choose a protocol from Table 3

***Qualitative** method verification*

Table 3 — Protocols to determine $eLOD_{50}$ and number of replicates needed per inoculation level

Protocol	Inoculation level of the test portion					
	High level $9 \times LOD_{50}$ / test portion	Intermediate level $3 \times LOD_{50}$ / test portion	Low level $1 \times LOD_{50}$ / test portion	3 cfu to 5 cfu /test portion	Blank	Total number of replicates
1	1	4	4	–	1	10
2	–	3	5	–	1	9
3	–	–	–	7	1	8
NOTE The abbreviation of colony forming units is cfu.						

- **Protocol 1:** uncertain of achieving level of contamination (inoculation with **culture**)
- **Protocol 3:** level of contamination is known *(inoculation with **reference** material)*
- **Protocol 2:** use if 1st choice of protocol didn't work, and need to **repeat** the experiment

Determine results

Qualitative method verification

High level: 9 × 5,7 = 54 cfu/test portion

Intermediate level: 3 × 5,7 = 18 cfu/test portion

Low level: 1 × 5,7 = 6 cfu/test portion

Table 6 — Determination of $eLOD_{50}$ based on the number of positive results per level of contamination using protocol 1

High inoculation level targeted 9 × LOD_{50}/ test portion	Intermediate inoculation level targeted 3 × LOD_{50}/ test portion	Low inoculation level targeted 1 × LOD_{50}/ test portion	Blank level	$eLOD_{50}$ cfu/test portion
1/1	4/4	4/4	0/1	< 1,0 × LIL[a]
1/1	4/4	3/4	0/1	= 0,5 × LIL
1/1	4/4	2/4	0/1	= 0,7 × LIL
Inoculum (cfu) at each level				**0,5 × 6 (LIL)**
54	**18**	**6**		**$eLOD_{50}$ = 3,0**

[a] LIL: Low inoculation level

Acceptability limits & results

Qualitative method verification

Table 16 — Acceptability limits for the verification of validated methods

Method	Performance characteristics	Acceptability limits
Qualitative	$eLOD_{50}$	For protocols 1 and 2: $eLOD_{50} \leq 4 \times LOD_{50}$ For protocol 3: ≥ 6 out of 7 positive results
Quantitative	S_{IR}	$S_{IR} \leq 2 \times$ lowest S_R mean value[a] determined in the validation study
	eBias	| $\log_{10}$ cfu/ml (inoculum) – mean $\log_{10}$ cfu/test portion (artificially contaminated [food] item) | ≤ 0,5 $\log_{10}$ for each of the inoculation levels
Confirmation or typing	inclusivity and exclusivity	100 % agreement between methods

[a] $S_{IR} \leq 2 \times S_R$ for validation studies with only one S_R value.

Acceptability limits:

$eLOD_{50}$ should be ≤ 4 × 5,7 (LOD_{50}) = 22,8 cfu

Implementation verification:

- $eLOD_{50}$ = **3,0** cfu ≤ **22,8** cfu
- **Meets acceptability limits**

(Food) item verification

Determine results

Qualitative method verification

High inoculation level = 9 cfu/test portion
Intermediate inoculation level = 3 cfu/test portion
Low inoculation level = 1 cfu/test portion

Table 6 — Determination of $eLOD_{50}$ based on the number of positive results per level of contamination using protocol 1

High inoculation level targeted 9 × LOD_{50}/ test portion	Intermediate inoculation level targeted 3 × LOD_{50}/ test portion	Low inoculation level targeted 1 × LOD_{50}/ test portion	Blank level	$eLOD_{50}$ cfu/test portion
1/1	4/4	4/4	0/1	< 1,0 × LIL[a]
1/1	4/4	3/4	0/1	= 0,5 × LIL
1/1	4/4	2/4	0/1	= 0,7 × LIL
Inoculum (cfu) at each level				**0,5 × 1 (LIL)**
9	**3**	**1**		**$eLOD_{50}$ = 0,5**

[a] LIL: Low inoculation level

(Food) item verification:

- $eLOD_{50}$ = **0,5** cfu ≤ **4** cfu
- **Meets acceptability limits**

Quantitative method verification *[Clause 6]*

Implementation verification

Implementation verification

Quantitative method verification

Intralaboratory reproducibility standard deviation (S_{IR}):

- Any (food) item within the scope of validation of the method
- S_{IR} determination is based on ISO 19036:2019
- Run the full procedure of the method as described, including the confirmation procedure for each test portion

Annex D provides guidance and examples on preparation of samples and test portions

Select (food) item: ISO 21528-2 *'Enterobacteriaceae'*

***Quantitative** method verification*

Implementation verification:

Category	Item	Characteristic
Chocolate, bakery and confectionary	Tiramisu	Validation study

10 samples:

- Different batches
- Manufacturers
- Other variations?

Implementation verification: S_{IR}

Quantitative method verification

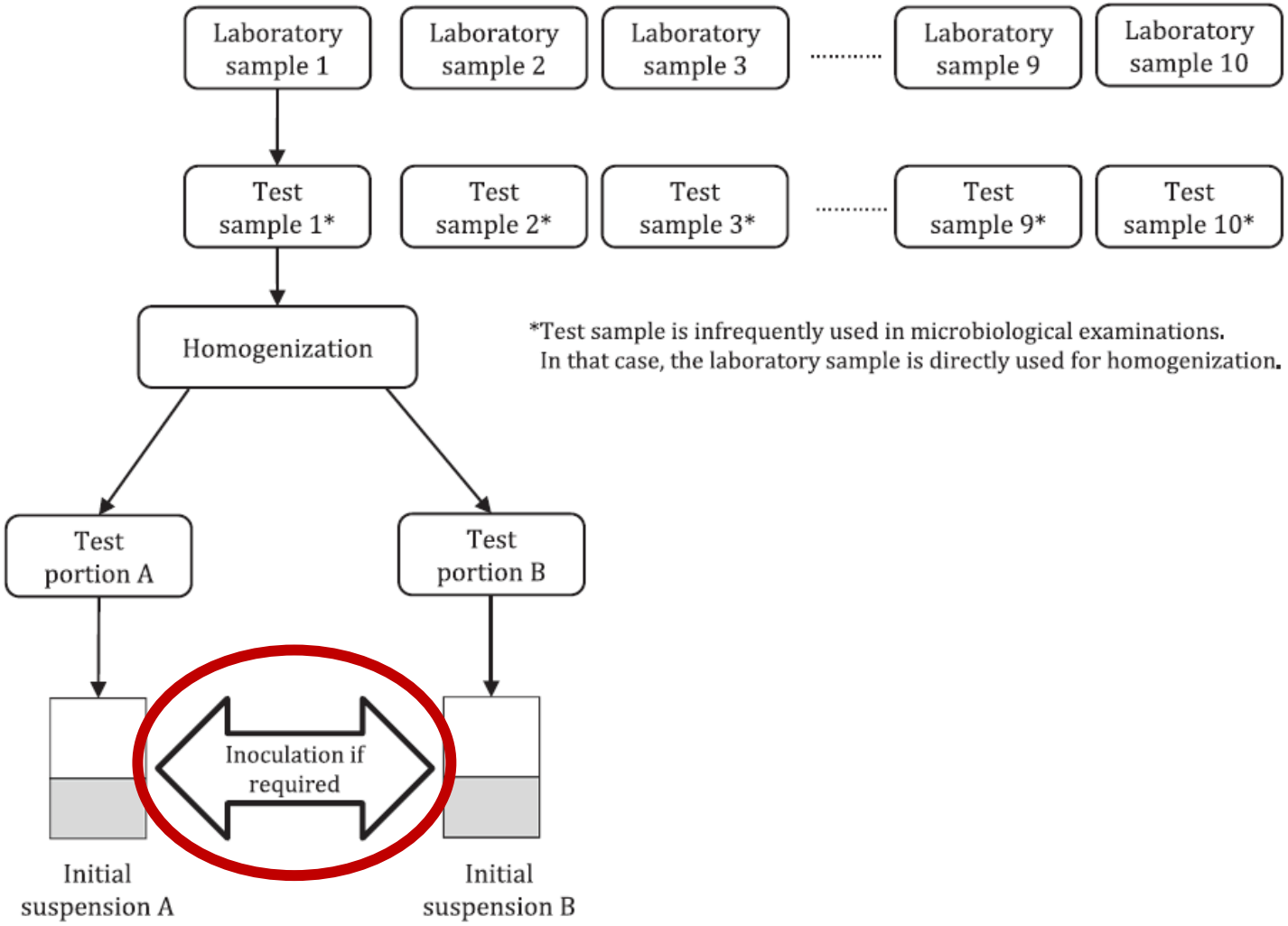


Figure D.1 — Preparation of samples for intralaboratory reproducibility standard deviation determination

Implementation verification: S_{IR}

Quantitative method verification

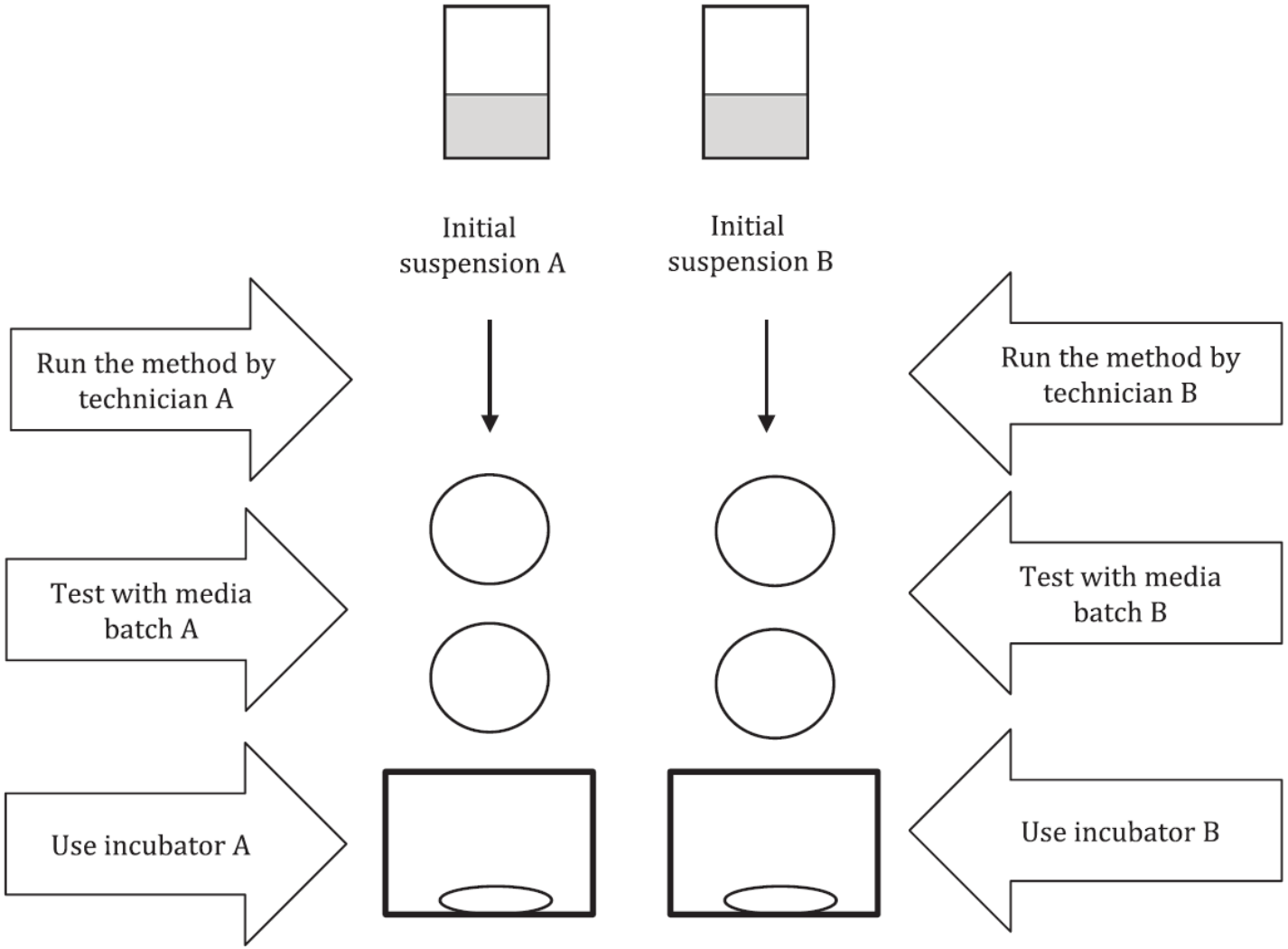


Figure D.2 — Suggestions for variations for intralaboratory reproducibility standard deviation determination

Implementation verification: S_{IR}

Quantitative method verification

Table 10 — Test results

Laboratory sample number	Expected contamination level cfu/g	Result A (x_{iA}) cfu/g	Result B (x_{iB}) cfu/g	$\log_{10}$ result A $y_{iA} = \log_{10}(x_{iA})$	$\log_{10}$ result B $y_{iB} = \log_{10}(x_{iB})$
1	30	< 40 (10)	< 40 (30)	≤ 1,60	≤ 1,60
2	300	110	182	2,04	2,26
3	300	410	620	2,61	2,79
4	600	640	330	2,81	2,52
5	600	690	570	2,84	2,76
6	600	780	640	2,89	2,81
7	600	620	1 300	2,79	3,11
8	600	870	1 500	2,94	3,18
9	6 000	8 600	6 400	3,93	3,81
10	6 000	16 000	5 000	4,20	3,70
11	6 000	> 15 000	13 400	> 4,18	4,13
12	30 000	20 000	32 000	4,30	4,51

Implementation verification: S_{IR}

Quantitative method verification

Table 11 — Calculation of S_{IR}

Laboratory sample number	$\log_{10}$ result A $y_{iA} = \log_{10}(x_{iA})$	$\log_{10}$ result B $y_{iB} = \log_{10}(x_{iB})$	Absolute difference $\lvert y_{iA} - y_{iB} \rvert$	Squared difference $\lvert y_{iA} - y_{iB} \rvert^2$
1	≤ 1,602 1	≤ 1,602 1	Not used	Not used
2	2,041 4	2,260 1	0,218 7	0,047 8
3	2,612 8	2,792 4	0,179 6	0,032 3
4	2,806 2	2,518 5	0,287 7	0,082 8
5	2,838 8	2,755 9	0,083 0	0,006 9
6	2,892 1	2,806 2	0,085 9	0,007 4
7	2,792 4	3,113 9	0,321 6	0,103 4
8	2,939 5	3,176 1	0,236 6	0,056 0
9	3,934 5	3,806 2	0,128 3	0,016 5
10	4,204 1	3,699 0	0,505 1	0,255 2
11	> 4,176 1	4,127 1	Not used	Not used
12	4,301 0	4,505 1	0,204 1	0,041 7
			Sum	0,650 0
			Sum/(2 × 10)	0,032 5
			$S_{IR} = \sqrt{(0,032\ 5)}$	0,18

— The calculated S_{IR} value of 0,18 is compared to the results of the validation study (data taken over from ISO 21528-2). Table 12 lists the S_R values obtained from that validation study.

$$s_{IR} = \sqrt{\frac{1}{2n}\sum_{i=1}^{n}\left(y_{iA} - y_{iB}\right)^2}$$

Implementation verification: S_{IR}

Quantitative method verification

Table 16 — Acceptability limits for the verification of validated methods

Method	Performance characteristics	Acceptability limits
Qualitative	$eLOD_{50}$	For protocols 1 and 2: $eLOD_{50} \leq 4 \times LOD_{50}$ For protocol 3: ≥ 6 out of 7 positive results
Quantitative	S_{IR}	$S_{IR} \leq 2 \times$ lowest S_R mean value[a] determined in the validation study
	eBias	| $\log_{10}$ cfu/ml (inoculum) – mean $\log_{10}$ cfu/test portion (artificially contaminated [food] item) | ≤ 0,5 $\log_{10}$ for each of the inoculation levels
Confirmation or typing	inclusivity and exclusivity	100 % agreement between methods

[a] $S_{IR} \leq 2 \times S_R$ for validation studies with only one S_R value.

Implementation verification: S_R values from validation study report

Quantitative method verification

Table 12 — Summary of S_R values from the validation study for ISO 21528-2

(Food) item	S_R values from the validation study			
	Low inoculation level	Intermediate inoculation level	High inoculation level	Mean value of three inoculation levels
Egg product	0,32	0,50	0,48	0,43
Raw meat	0,28	0,36	0,57	0,40
Animal feed	0,18	0,17	0,20	0,18
Pasteurized milk	0,24	0,18	0,19	0,20
Tiramisu	0,22	0,28	0,13	0,21

Acceptability limits: $S_{IR} \leq 2 \times$ *lowest* S_R *mean value*

- Lowest S_R mean value = 2 × 0,18 = **0,36**
- S_{IR} obtained in implementation verification study = **0,18**
- **0,18 ≤ 0,36**
- **Meets acceptability limits**

(Food) item verification

(Food) item verification: eBias

Quantitative method verification

Estimated bias (eBias):

1. Select (food) items

2. Artificially contaminate at 3 levels
   - Different laboratory sample or batch for each level
   - Each level performed in duplicate

3. Enumerate the contaminated (food) item and the inoculum

4. Test uninoculated test portion for each to determine background microbiota

(Food) item verification: inoculation of test portions

Quantitative method verification

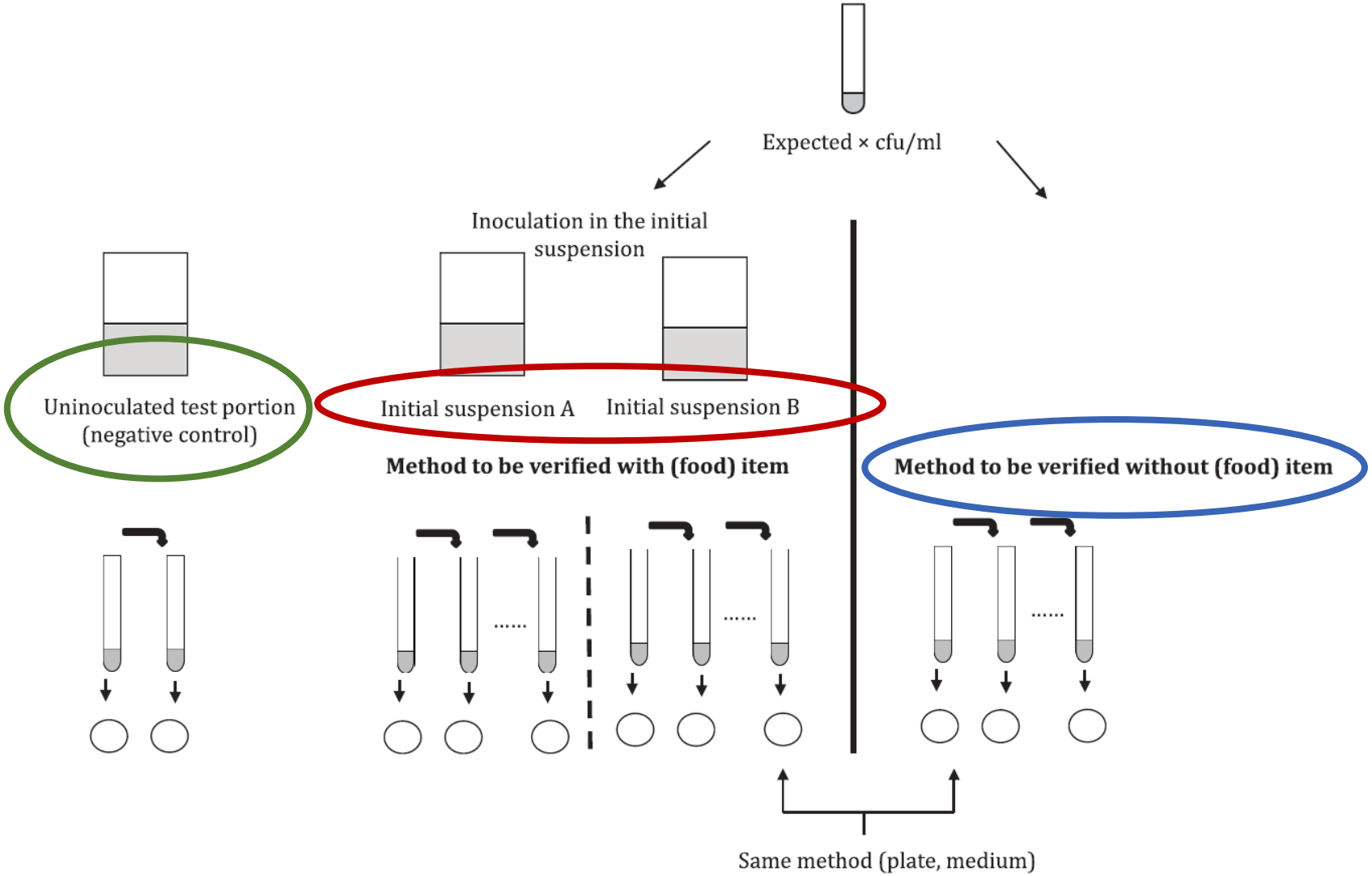


Figure D.6 — Example of quantitative method verification (eBias) using artificial contamination

(Food) item verification: eBias determination

Quantitative method verification

Table 16 — Acceptability limits for the verification of validated methods

Method	Performance characteristics	Acceptability limits
Qualitative	$eLOD_{50}$	For protocols 1 and 2: $eLOD_{50} \leq 4 \times LOD_{50}$ For protocol 3: ≥ 6 out of 7 positive results
Quantitative	S_{IR}	$S_{IR} \leq 2 \times$ lowest S_R mean value[a] determined in the validation study
Quantitative	eBias	| $\log_{10}$ cfu/ml (inoculum) – mean $\log_{10}$ cfu/test portion (artificially contaminated [food] item) | ≤ 0,5 $\log_{10}$ for each of the inoculation levels
Confirmation or typing	inclusivity and exclusivity	100 % agreement between methods

[a] $S_{IR} \leq 2 \times S_R$ for validation studies with only one S_R value.

(Food) item verification: eBias determination

Quantitative method verification

Table 13 — Test results obtained using the method to be verified

Level		Mean result: Artificially contaminated (food) item (log_{10} cfu/g or ml)[a]	For comparison: Result Artificially contaminated (food) item (log_{10} cfu/test portion)[a]	For comparison: Result Inoculum suspension [without (food) item] (log_{10} cfu/ml)	eBias: absolute difference in results between artificially contaminated (food) item per test portion and the inoculum suspension	≤ 0,5 log_{10} cfu/ml
10^1	Laboratory sample 1 (from batch 1), test portion 1	2,06 (average of 1,87 and 2,25)	3,06	3,17	0,11	Meets
	Laboratory sample 1 (from batch 1), test portion 2					
10^3	Laboratory sample 2 (from batch 2), test portion 1	3,11 (average of 3,16 and 3,06)	4,11	4,05	0,06	Meets
	Laboratory sample 2 (from batch 2), test portion 2					
10^5	Laboratory sample 3 (from batch 3), test portion 1	3,99 (average of 3,93 and 4,04)	4,99	5,29	0,30	Meets
	Laboratory sample 3 (from batch 3), test portion 2					

[a] This example is based on the use of a 10-gram test portion inoculated with 1 ml of inoculum.

Validated alternative confirmation and typing methods – Technical protocol for verification *[Clause 7]*

Confirmation and typing method verification require only implementation verification

- Review method validation data
- Choose 1 selective agar plate used in the validation study
- Use this agar to perform implementation verification
  - If no selective agar plate was tested, select and use one non-selective agar plate tested during the validation study

Annex E provides guidance and examples for confirmation and typing method verification

Selection of strains

Confirmation and typing *method verification*

Table 14 — Number of strains for implementation verification of validated alternative confirmation or typing methods

Level of the confirmation	Inclusivity study	Exclusivity study
Family	5	5
Genus		
Species		
Microbial (sub)type (e.g. serotyping of *Salmonella*)		

Acceptability limits

Confirmation and typing method verification

Table 16 — Acceptability limits for the verification of validated methods

Method	Performance characteristics	Acceptability limits
Qualitative	$eLOD_{50}$	For protocols 1 and 2: $eLOD_{50} \leq 4 \times LOD_{50}$ For protocol 3: ≥ 6 out of 7 positive results
Quantitative	S_{IR}	$S_{IR} \leq 2 \times$ lowest S_R mean value[a] determined in the validation study
	eBias	| $\log_{10}$ cfu/ml (inoculum) – mean $\log_{10}$ cfu/test portion (artificially contaminated [food] item) | ≤ 0,5 $\log_{10}$ for each of the inoculation levels
Confirmation or typing	inclusivity and exclusivity	100 % agreement between methods

[a] $S_{IR} \leq 2 \times S_R$ for validation studies with only one S_R value.

Example: overview of verification results *[see Table E.1]*

Alternative confirmation method verification

Tested strains	I/E*	Characteristics of the strain	Expected result	Result	Interpretation
1	I	*L. monocytogenes* (serotype 4b) WDCM 00021 Human isolate	Positive	Positive	Agreement
2	I	*L. monocytogenes* (serotype 1/2a) WDCM 00109 Guinea-pig isolate	Positive	Positive	Agreement
3	I	*L. monocytogenes* (genotype IV) 12MOB112LM Meat isolate	Positive	Positive	Agreement
4	I	*L. monocytogenes* (genotype II) 12MOB118LM Dairy isolate	Positive	Positive	Agreement
5	I	*L. monocytogenes*, Field strain LM01 Smoked salmon isolate	Positive	Positive	Agreement
6	E	*L. innocua* WDCM 00017	Negative	Negative	Agreement
7	E	*L. ivanovii* WDCM 00018	Negative	Negative	Agreement
8	E	*Bacillus cereus* WDCM 00001	Negative	Negative	Agreement
9	E	*Enterococcus faecalis* WDCM 00009	Negative	Negative	Agreement
10	E	*Staphylococcus aureus* WDCM 00034	Negative	Negative	Agreement

***I/E =** inclusivity / exclusivity

Protocol for the verification of non-validated reference methods in a single laboratory *[Annex F]*

Scope of Method *vs* ~~Validation~~ *vs* Laboratory application

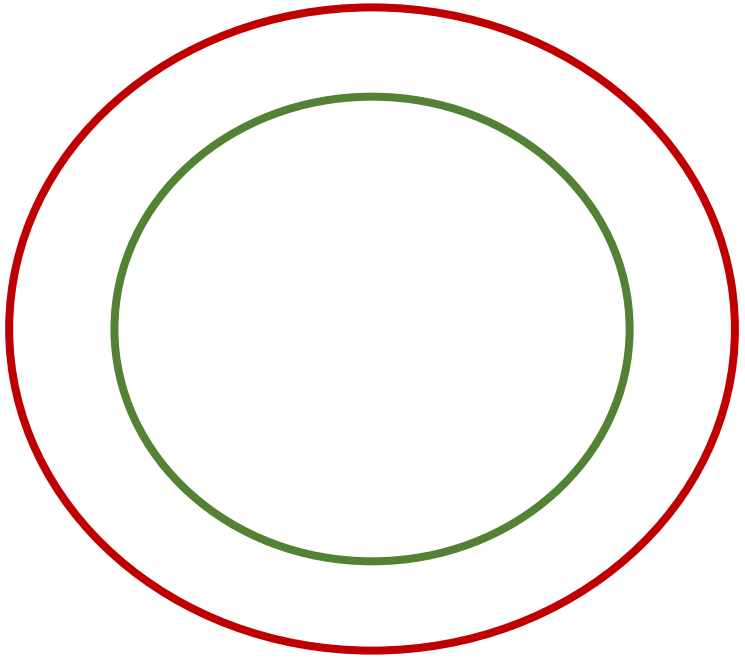
***Non-validated** reference methods*

Method

It specifies the (group of) products (categories or types or items) for which the method is claimed to be *applicable*

Laboratory

It specifies the (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



(Food) item verification

***Non-validated** reference methods*

Demonstrate the competence of the user laboratory to perform the **non-validated reference method** with **(food) items that are tested in the user laboratory**

[no implementation verification – because there is no validation study]

The user laboratory shall:

- select 1 non-challenging (food) item from a (food) category claimed in the scope of the reference method

- select 1 challenging (food) item from each (food) category, claimed in the scope of the reference method, that is also under the scope of the laboratory application

Annex F: Protocol for the verification of non-validated reference methods in a single laboratory

Summary of acceptability limits

Non-validated *reference methods*

Table F.5 — Acceptability limits for the verification of non-validated reference methods

Method	Performance characteristics	Acceptability limits
Qualitative	$eLOD_{50}$	For protocols 1 and 2: $eLOD_{50} \leq 4$ cfu/test portion For protocol 3: ≥ 6 out of 7 positive results
Quantitative	eBias	| $\log_{10}$ cfu/ml (inoculum) – mean $\log_{10}$ cfu/test portion (artificially contaminated [food] item) | ≤ 0,5 $\log_{10}$ for each of the inoculation levels

Transition document for implementation of ISO 16140-3

Transition period for implementation: general principles

The transition arrangement is as follows:

- **until 2027-12-31**, user laboratories may perform method verification of non-validated reference methods and in accordance with ISO 16140-3, Annex F
- **from 2028-01-01**, only validated reference methods are applicable for method verification

After this date, reference methods (including ISO or CEN standards) shall be validated before a verification can be performed in accordance with ISO 16140-3

Reminder:

- ISO standards are *voluntary* documents
- ISO develops standards but has *no authority* over their implementation

Transition period: different situations

Methods <u>already accredited</u> under the scope of laboratory application:

- do not need to re-verify, unless changes made to the method

Methods or (food) categories <u>new</u> to the scope of laboratory application:

- verify methods introduced to the laboratory after publication of ISO 16140-3
- verify new (food) category additions to accredited methods under scope of laboratory application

Methods <u>revised</u> after they have been accredited under the scope of laboratory application:

- Depends - major or minor change, as determined by the certification body


thank you
danke
gracias
merci
obrigado
bedankt
dziękuję
teşekkür ederim
go raibh maith agat
ngiyabonga
謝謝
рахмат
Баярлалаа
спасибо
спасибі
faafetai lava
vinaka
blagodaram
dank je
misaotra
matondo
paldies
grazzi
mahalo
tapadh leat
хвала
asante
manana
obrigada
tenki
murakoze
mochchakkeram
djiere dieuf
tau
дякую
mamnun
chokrane
mersi
kia ora
barka
welalin
tack
spas
kiitos
dankie
dhanyavad
nanni
nandri
mauruuru
köszönöm
hvala
gràcie
bayarlalaa
enkosi
akun dankon
aĉiū
chnorakaloutioun
gratias ago
gràcies
sulpáy
taiku
sobodi
dēkuji
sukriya
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arigatô
takk
dakujem
trugarez
mèsi
didi madloba
sagolun
najis tuke
ありがとう
tanemirt
rahmet
grazie
dhanyavadagalu
shukriya
mercé
мерси
kam sah hamnida
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terima kasih
감사합니다
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Public website of ISO/TC 34/SC 9 ‘Microbiology’

- Information: method validation and verification
- Background: six parts of ISO 16140 series

Supporting materials*

- Transition document: implementation of ISO 16140-3
- Excel®-based program for assistance on statistics
- Recording of this webinar

Presentations:

- Overview of the entire ISO 16140 series
- Overview of ISO 16140-3 (today’s presentation)
- “Deep-dive training” on ISO 16140-3

***All these materials will be available before mid-March on the SC 9-website.**

https://committee.iso.org/home/tc34sc9

Presented by ISO/TC 34/SC 9/WG 3 ‘Method validation’

Method validation and method verification

21 October 2020

The ISO 16140 series is dedicated to the validation and verification of microbiological methods. These International Standards are designed to help food and feed testing laboratories, test kit manufacturers, competent authorities, and food and feed business operators to implement microbiological methods.

Learn more about ISO 16140 series, and the necessary stages of validation and verification of methods before use.

Development of the ISO 16140 series

The ISO 16140 series consists now of six parts with the general title, *Microbiology of the food chain - Method validation*:

Questions?

Closing words