New ISO reference and alternative methods. Impact on food microbiology laboratories

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Introduction

According European Regulation EC 2073/2005 on microbiological criteria for foodstuffs, food business operators shall perform testing against the microbiological criteria when validating or verifying the correct functioning of the procedures based on HACCP and Good hygiene practice.

The EN/ISO analytical methods included in the regulation shall be applied as the reference methods. However, food business operators should have the possibility to use alternative analytical methods other than the reference methods, if the alternative methods are validated against the reference method in accordance with the protocol set out in EN ISO 16140.

The new ISO/EN reference methods, developed and validated by a multiple stakeholder collaboration and consensus, have been optimized and, in some cases simplified allowing a reduction of workload, costs and time to results compared with previous versions, without compromising the validity of the results.

Performance criteria included in the new standards brings also valuable information. Reference values (e.g. LOD, Repeatability, reproducibility) are now available to compare against when the method is implemented in a quality control laboratory.

Last but not least, new methods have a direct impact on alterative methods validated against these standards. When changes in the standard are considered major with significant effect on method performance, re-validation and new verification should be conducted to guarantee results from the samples analysed are still valid.

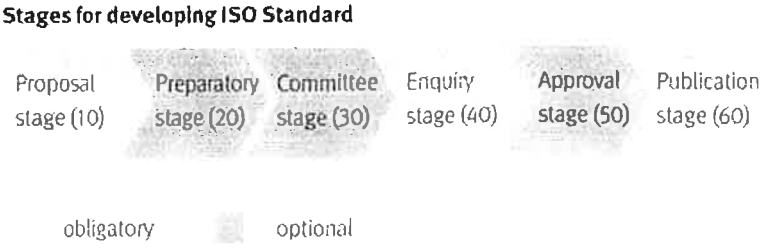
Development and benefits from reference methods.

ISO/TC34/SC9 and CEN/TC275/WG6 are the groups responsible to propose the development, update or withdraw of reference standard methods in food microbiology.

ISO/TC34/SC9 manages 78 published standards and 20 standards under development. It includes 32 participant members and 32 observing members worldwide. Subcommittee includes 29 working groups.

CEN/TC275/WG6 includes 33 European countries.

The development of ISO methods is a long journey requiring several steps for approval:



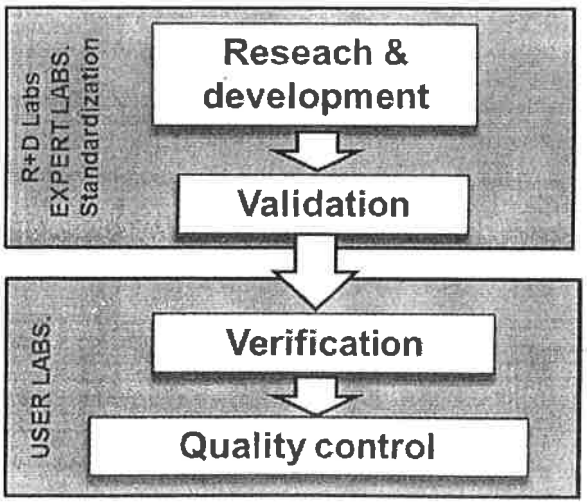
The use of ISO methods as a reference is preferred because are:

- Internationally recognized.
- Included in regulations (less disputes).
- Easy to accredited.
- Worldwide use (accessible).
- Using non-proprietary technology.
- Based on consensus and global expert opinion.
- Developed through a multi-stakeholder process.

Implementation reference methods

In general, two stages are needed before a method can be used in a laboratory (ISO/DIS 16140-3:2017)

1. The first stage is the **validation** of the method. This is either conducted in several laboratories or in one laboratory.
2. The second stage is method **verification**, where a laboratory demonstrates that it can satisfactorily perform a validated method.



Validation data included in reference methods, provide performance characteristics to demonstrate the method is fit for purpose, but also to guarantee a good implementation when is going to be verified by the user laboratory.

Some examples of the different performance characteristics available in the new published methods for both detection and enumeration methods are:

Example of enumeration methods from ISO 11290-2:2017: Enumeration *Listeria monocytogenes*

Table E.1 — Results of data analysis obtained with cold-smoked salmon

Parameter	Cold-smoked salmon (contamination level of *Listeria monocytogenes*)		
	110 cfu/g (low level)	1 300 cfu/g (medium level)	13 000 cfu/g (high level)
Number of participating collaborators	15	15	15
Number of collaborators retained after evaluation of the data	15	14	15
Number of samples tested	30	30	30
Number of samples retained after evaluation of the data	30	28	30
Mean value Σa ($\log_{10}$ cfu/g)	1,85	3,04	3,95
Repeatability standard deviation s_r ($\log_{10}$ cfu/g)	0,13	0,10	0,12
Repeatability limit r:	—	—	—
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,37	0,28	0,32
as ratio on normal scale (cfu/g)	2,33	1,92	2,10
Reproducibility standard deviation s_R ($\log_{10}$ cfu/g)	0,18	0,19	0,19
Reproducibility limit R:	—	—	—
as difference on $\log_{10}$ scale ($\log_{10}$ cfu/g)	0,51	0,53	0,54
as ratio on normal scale (cfu/g)	3,26	3,37	3,44

Example for detection methods from ISO 22964:2017: Detection of *Cronobacter* spp

Table D.1 — Results of data analysis obtained with milk based powdered infant food formula

Performance characteristic	Blank	Low inoculation level[a] 4 cfu/10 g	High inoculation level[a] 55 cfu/10 g
Number of participating collaborators	17	17	17
Number of collaborators retained after evaluation of the data	15	15	15
Number of samples per lab	8	8	8
Number of samples retained after evaluation of the data	120	88	120
Sample size (g/ml/cm²/item)	10	10	10
Sensitivity %	-	-	100
Specificity %	99	-	-
LOD_{50}, (95 % confidence interval) cfu/test portion	-	1,1 (0,8 to 1,4)	

[a] Milk based powdered infant formula (commercially available), containing bifidobacteria at a level between 1,0 E+6 cfu/g and 1,0 E+7 cfu/g, was inoculated with *Cronobacter sakazakii*.

It is important to consider the ISO detection methods have been validated for a maximum test portion (e.g. 10g, 25 g). A smaller test portion may be used without the need for additional validation/verification provided that the same ratio between (pre-)enrichment broth and test portion is maintained. A larger test portion than that initially validated may be used if a validation/verification study has shown that there are no negative effects on the detection of Salmonella spp. Validation can be conducted according to the appropriate parts of ISO 16140. Verification for pooling samples can be conducted according to the protocol described in ISO 6887-1:2017, Annex D.

In order to evaluate the impact of the new reference methods compared with previous versions, changes are classified in the introduction of each respective standard as:

Major change = technical change in the method that is expected to produce a different result.

From the implementation in routine laboratories point of view, the activities to implement a method with major changes can include:

- Evaluation of new media/supplier (ISO 11133).
- Acquisition of new strains.
- Re-validation against new standard (only for alternative methods).
- Implementation new method version.
- Beta-test.
- Lab accreditation: new assessment may be required.
- Verification.
- Adapt templates.
- Internal quality control/Method monitoring.

Minor change = editorial change or a minor technical change in the method that is not expected to affect the result obtained with the method.

From the implementation in routine laboratories point of view, the activities to consider could be:

- Implementation new method version.
- Adapt templates.
- Internal quality control/Method monitoring.

Despite the previous classification, a change considered as major in the technical content of the method can be considered to be minor because no significant effect on the method performance characteristics or test results was observed.

Summary of reference methods published in 2017:

ISO Number	Microorganism/ toxin. Method description	Technical changes
10272-1; 10272-2	***Campylobacter spp.*** Part 1: Detection by enrichment or direct isolation. Part 2: Plate enumeration.	Minor changes.
18465	**Cereulide** (*B. cereus* emetic toxin). Quantitative analysis using HPLC or UHPLC connected to LC-MS/MS.	New method.
22964	***Cronobacter* spp.** Detection by enrichment and isolation.	Major changes: enrichment improved, scope enlarged.
16654/ Amd.1	***E. coli* O157** Detection by enrichment and isolation.	Minor changes: only inter-laboratory results included.
21528-1; 21528-2	***Enterobacteriaceae*** Part 1: Detection by enrichment and isolation. Enumeration by MPN (informative annex). Part 2: Plate enumeration.	Major changes: Enrichment reduced from 2 to 1 day. New confirmatory tests. Part 2: Minor changes
11290-1; 11290-2	***Listeria monocytogenes* and *Listeria* spp.** Part 1: Detection by enrichment and isolation. Part 2: Plate enumeration	Minor changes: Enrichment reduced from 48 to 24 h, simplification for confirmatory steps.
6579-1	***Salmonella spp.*** Detection by enrichment and isolation.	Minor changes.
19020	**Staphylococcal enterotoxins** (SEA to SEE) Detection by extraction and dialysis + ELISA using commercially available detection kits.	New method.
21872	***Enteropathogenic Vibrio*** *(V. parahaemolyticus, V. cholerae and V. vulnificus.* Detection by enrichment and isolation.	Major changes: Target *V. vulnificus*. Optional molecular identification methods.
15216	**Enteric viruses** (HAV and norovirus GI and GII). Quantification on soft fruit, leaf, stem and bulb vegetables and food surfaces by RNA extraction and amplification real-time RT-PCR	Minor changes.
10273	***Yersinia enterocolitica.*** *Detection by enrichment and isolation*	Major changes: Confirmation of pathogenic *Y. enterocolitica*, tests added. Changes in inoculation, isolation, incubation times.