

Update on ISO & CEN food microbiology methods

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ISO/TC 34/SC 9

Microbiology of the Food Chain

- ▶ Committee has approx. 100 publications, >10% of all TC34 subcommittees
- ▶ Now 38 working groups (WGs), 7 *ad hoc* working groups, 1 subgroup WG2/WG3
- ▶ New WGs - sampling techniques (WG35), LAMP requirements(WG36), PCR (WG37), STEC (WG38)
- ▶ Considerable focus on validation and organising inter-laboratory studies (ILS)
- ▶ Further development on SC9 website
<https://committee.iso.org/home/tc34sc9>

ISO 7218: 2024

- ▶ Calculations and expression of results simplified
- ▶ Three excel tools for results calculation added (plate count and MPN)
- ▶ Equipment section reorganized into groups with similar purposes and requirements to reduce repetition
- ▶ Cross-references to other general microbiology standards added to reduce repetition (ISO 11133, ISO 16140 series, ISO 19036)
- ▶ Information on laboratory quality control, characterization of control microorganisms and confirmatory tests added.
- ▶ Information on spiral plating expanded

ISO 7218: Further information

- ▶ The online colony count techniques (CCT) calculator cited in clause 11 is available at: <https://standards.iso.org/iso/7218/ed-4/en>
- ▶ Downloadable zipfile containing excel documents for counting colonies and their acceptability limits, a general MPN program for MPNs and a separate shellfish calculator with their SDs, CIs and rarity values.
- ▶ Clause 16 Laboratory quality control describes factors affecting validity of test results, various controls, replicate testing, spiked samples, control charts, external quality assessment
- ▶ Clause 17 describes performance characteristics, validation and verification for enumeration, detection and confirmation methods.
- ▶ Annex B describes how to calculate the confidence intervals (CIs) for counting techniques, which can be used to characterize the microbial dispersion (with a 95% probability) if the value of uncertainty is not available. Also contains examples, tables for weighted means and CIs and a table of CIs for low numbers of colonies (1 - 30).

ISO 7218: 2024 Spiral Plating

- ▶ This standard now describes spiral plating in full
- ▶ Description, general use and maintenance are described in 6.4.3
- ▶ Surface plating using a spiral plater described in 11.2.3.3
- ▶ Counting of colonies for spiral plating described in 11.2.6.2
- ▶ Calculation of colony count from spiral plater are described in 11.2.7.3
- ▶ Procedure will be removed from ISO 4833 when revised
- ▶ ISO 7218:2024 supersedes ISO 4833-2 for spiral plating

ISO 11133

Performance testing of culture media

- ▶ Currently finishing the revision for the DIS stage
- ▶ Annex L on pH measurement of culture media added
- ▶ Annex M Guideline for the evaluation of buffered peptone water added (from CEN 463 WG5)
- ▶ Annex N Reference Culture media added
- ▶ Annex O Guideline for the preparation of standardized suspensions (inocula) and preservation by ultra-low freezing added
- ▶ Revision of 6.4 on general requirements for microbiological performance testing (tabular form)
- ▶ Revision of Annex G to align with NEN 6603; now informative

ISO 16140 (validation)

Part 1 Terminology	Revision started late 2023
Part 2 Validation of alternative (proprietary) methods against a reference method	Amendment 1: Revision of qualitative method comparison study data evaluation, relative level of detection calculations in the interlaboratory study, calculation and interpretation of the relative trueness study, and inclusion of a commercial sterility testing protocol for specific products' published 2024 Full revision started 2024
Part 3 Verification of reference and alternative methods in the laboratory	"Frequently Asked Questions" in relation to implementation of part 3 now posted on WG3 webpage of SC9 website
Part 4 In-house (single laboratory) method validation	Amendment 1 dealing with a new Annex H - validation of larger test portion size for qualitative methods; published September 2024
Part 5 Factorial inter-laboratory method validation	-
Part 6 Validation of microbiological confirmation methods	Systematic review currently ongoing
Part 7 Protocol for the validation of identification methods of microorganisms	Publication expected Dec 24/ Jan 25
ISO 17468 technical	Revision published Nov. 2023

ISO 16140 (cont)

- ▶ 16140 part 8: Viruses and Parasites 2nd draft ISO/NP out now (internal only)
- ▶ 16140 part 9: Bacterial toxins and biogenic amines Work begun, registered as a Preliminary Work Item (PWI)
- ▶ 16140-2 revision will focus on alignment between ISO and AOAC, classification of sample types and commercial sterility testing for a broad range of foods.
- ▶ WG3 also drafted templates for design of validation of both qualitative and quantitative methods
- ▶ Planning a joint webinar between ISO and ILAC (accreditation) on method verification for technical assessors.
- ▶ Request to validation organisers to include larger portion sizes for qualitative methods if possible.

ISO 19036

Measurement Uncertainty

- ▶ Excel tool revised and verified, will be uploaded to SC9 website shortly
- ▶ Subgroup formed to develop a database on matrix uncertainty - values to be provided on request

ISO 4833

Enumeration of microorganisms at 30 °C

- ▶ Completed study comparing pour and spread plating
- ▶ Agreement that results were comparable, differences were small and not of practical importance
- ▶ Agreement that two parts would be combined
- ▶ Revision begun
- ▶ Validation data already available
- ▶ New Annex summarising comparison report
- ▶ New Annex containing validation data and performance characteristics
- ▶ Removal of spiral plating

ISO 6887-1: 2017 + A1:2024

Preparation of test samples, initial suspension and decimal dilutions

- ▶ Provides definitions for compositing and pooling
- ▶ Describes requirements for larger portion size -
pre-warming of enrichment broth,
checking time to reach incubation temperature for frozen, refrigerated and
room temperature samples,
ensuring at least 16 h at specified incubation temperature
- ▶ Updated Annex A on compositing and pooling procedures
- ▶ Removed information in Annex D on validation of larger test portion size for
qualitative methods to ISO 16140-4:2024 Annex H, leaving only a note with
cross-reference in Annex D
- ▶ Note: currently considering spacing between pre-enrichment/ enrichment
bags

ISO 6887 - other parts

- ▶ 6887-2: (Meat) No activity
- ▶ 6887-3: (Seafood) Call for project leader to revise this
- ▶ 6887-4: (Other foods) active WG, call for feedback on food additives, nutraceuticals, functional foods, proprietary foods.
- ▶ 6887-5: (Dairy) WG for 6887-4 will also call for information on processing butter samples (2 procedures described in 6887-5).
- ▶ 6887-6: Needs revision, insufficient expertise available

ISO 11290

Listeria monocytogenes and *Listeria* spp.

- ▶ Amendment for both parts (late 2025?)
- ▶ Replacement of *L.monocytogenes* WDCM 00109 by WDCM 00231 (available as DSM 112143/JCM 35698/ NCTC 14697) for performance testing of media.
- ▶ Addition of storage specifications: Before analysis store at 2 °C - 8 °C (all unfrozen samples), if frozen defrost according to ISO 7218. Examine samples immediately or within 8h of receipt. If stored overnight, store at <5 °C for up to 24h.

ISO 11290

Improvement of enrichment studies

- ▶ Preliminary comparative studies using naturally contaminated and spiked samples for pre-enrichment step; 15 laboratories from 11 countries took part using diverse sample types
- ▶ Compared current ½ Fraser method with FDA BAM method, UVM1 Canadian method, 6 commercially available methods and a modified ½ Fraser broth
- ▶ 3 commercially available methods significantly more sensitive than ISO 11290 method

ISO 11290 -1

- ▶ LOD study ongoing using Neogen /LESS+, BIO-RAD /LSB and Thermofisher 24 LEB as pre-enrichment
- ▶ Comparison studies for improving enumeration to lower the limit to 1 cfu/g: will use Norwegian approach
- ▶ Subgroup on analysis of silage

Different approaches tried such as addition of buffers, comparison of alternative broths or sample preparation.

ISO 15213

Detection and enumeration of sulphite reducing clostridia and *C.perfringens*

- ▶ Part 1 - Enumeration of sulphite -reducing *Clostridium* spp. - published 2023. Amendment under way to implement several technical corrections and add improvements to tables C1 - C7.
- ▶ Part 2 - Enumeration of *Clostridium perfringens* by colony count technique - published 2023. Uses TSC agar, with confirmation by acid phosphatase production or use of sulfite motility agar (SIM - not yet available commercially). Amendment in preparation, which will include updating of Annex D on *C.perfringens* toxinotyping (by PCR).
- ▶ **NB: acid phosphatase reagent contains Fast Blue salt which is toxic and carcinogenic (requires risk assessment).**
- ▶ Part 3 - Detection of *Clostridium perfringens* - published 2024 as TS as too few participating laboratories in validation. Growth in Rapid Perfringens Medium RPM incubated 46 °C/18h. Subcultured to surface of TSC agar and lactose egg yolk neomycin agar (LENA; not available commercially). Amendment in preparation to address further technical changes.

Detection and enumeration of *Escherichia coli*

- ▶ WG18 now encompasses ISO 11866 and ISO 7251 as well as ISO 16649.
- ▶ Revision of Part 1 of ISO 16649 already published.
- ▶ An inter laboratory study protocol has been prepared to compare the use of spread and pour plating techniques for part 2. The DIS will be launched after the ILS.
- ▶ Validation of all parts of 16649 and ISO 7251 is needed.
- ▶ Part 1 of ISO 11866 will be harmonised with ISO 16649-3
- ▶ The CD ballot of ISO 7251:2005/DAMD 1 to include performance testing of culture media will be launched shortly.

Escherichia coli

ISO 16649 -1, -2, -3, and ISO 7251

- ▶ ISO 16649-2: ongoing comparison study of pour v. spread plates - to be completed by end 2024
- ▶ Once complete, validation studies will be carried out
- ▶ Performance data for previous *E.coli* methods to be reviewed
- ▶ Consideration ongoing whether 3 different MPN methods needed -
ISO 16649-3, ISO 7251, ISO 11866-1 (dairy)

(Growth in MMGM/subculture to TBX, growth in LT broth /subculture to EC broth, and growth in LT broth containing MUG and 0.01% tryptophan/ fluorescence and indole production respectively)

ISO 22174:2024

PCR for detection and quantification of microorganisms - general requirements and definitions (includes viruses)

- ▶ Supersedes 2005 version
- ▶ Includes requirements for the implementation of digital PCR
- ▶ Includes requirements for monitoring of laboratory flows and environmental monitoring for PCR (Clause 9)
- ▶ Extends section 12.2.2 control reaction with descriptions of different controls
- ▶ Change of 12.3 to include quantitative evaluations
- ▶ Includes Clause 14 on validation and verification

ISO 7932:2004

Enumeration of *presumptive Bacillus cereus*

- ▶ New convenor appointed to WG 20, revision process re-started
- ▶ Wish to improve enumeration by replacing MYP with chromogenic medium
- ▶ US/FDA conducted single laboratory validation comparing BACARA and MYP with BioMerieux (BACARA2) and Biorad (RAPID).
- ▶ Wish to run a comparative study of BACARA2, RAPID, Biokar (COMPASS) and possibly one other medium.
- ▶ Must have an “open” formula
- ▶ Ingredients must be widely available
- ▶ Selected isolation agar should also promote *B.cytotoxicus* growth

ISO 6888 series

Enumeration of coagulase positive staphylococci (*S.aureus* and other species)

- ▶ Revision of all parts published 2021; parts 1 and 2 deemed equivalent
- ▶ Amendment 1:2023 published September 2023 for parts 1 and 2 to correct 2021 documents
- ▶ Currently finishing protocols for ILS validation studies for all three parts; some validation data already available

