International Association for Food Protection®

WEBINAR

What Are the Standard Methods And What Makes Them So Special

Thomas Hammack, U.S. Food and Drug Administration
Paul in 't Veld, Netherlands Food and Safety Authority

May 11, 2018 9:00 a.m. Central Time

International Association for Food Protection
WEBINAR

Webinar Housekeeping

- Audio is being transmitted over the computer so please have your speakers ‘on’ and volume turned up in order to hear. A telephone connection is not available.
- During the session you can submit questions via the **Q & A section** in the **Chat Box** Questions will be answered at the end of the presentation.
- This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org.

Moderators

- Stephanie Pollard, Clear Labs
- Omar A. Oyarzabal, University of Vermont Extension

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IAFP
Applied Laboratory Methods PDG

THE UNIVERSITY OF VERMONT
EXTENSION

University of Vermont Extension

and

IAFP Applied Laboratory Methods
Professional Development Group

Presenters

Thomas Hammack

- United States Food and Drug Administration
- Supervisory Microbiologist
- Chair of FDA's Microbiology Methods Validation Subcommittee
- Past Chair of FDA's Bacteriological Analytical Manual (BAM) Council and current member
- Active in ISO and AOAC International

Presenters

Paul in ’t Veld

- Food Microbiologist at Netherlands Food and Safety Authority
- Active in standardization of methods for International Organization for Standardization (ISO) and Comité Européen de Normalisation (CEN)
- Chair of ISO Working Group 3 (WG3) for method validation standards (ISO 16140 series)

Bacteriological Analytical Manual

U.S. Food and Drug Administration

- One of the oldest regulatory agencies in U.S.
- Food and Drugs Act of 1906
- Federal Food, Drug and Cosmetic Act, 1938
- Regulated products make up 22% of all consumer expenditures
- Approximately 10% of every consumer dollar spent on food

Regulatory Authority

- **Responsible for the safety of 80% of all food consumed in the United States**
  - **Entire domestic and imported food supply**
  - **Except**
    - **Meat**
    - **Poultry**
    - **Frozen, dried and liquid eggs**

Regulated by USDA

Bacteriological Analytical Manual (BAM)

- **A compendium of methods used by FDA for the regulatory analysis of foods**
- **Intended as a vehicle for information and standardization within FDA when first established in 1965**
- **Published by AOAC International from 1976 – 1998**
- **Published as the *BAM online* by FDA and ceased to exist as a paper document in 2000**

Bacteriological Analytical Manual

- **Divided into 6 sections—**
  - **General guidelines/procedures**
  - **Methods for specific pathogens**
  - **Methods for microbial toxins**
  - **Molecular methods for foodborne pathogens**
  - **Additional methods**
  - **Appendixes**

Bacteriological Analytical Manual

- **Methods for Specific Pathogens**
  - **Diarrheagenic *Escherichia coli* (including EHEC)**
  - ***Salmonella***
  - ***Shigella***
  - ***Listeria monocytogenes***
  - ***Clostridium botulinum***
  - **Yeasts, Molds, and Mycotoxins**

Bacteriological Analytical Manual

- **Characteristics**
  - **Mostly cultural methodologies that produce isolates**
  - **Methods that are long established**
  - **Many methods are time consuming and laborious**

Bacteriological Analytical Manual

- **Characteristics**
  - **FDA relies on the BAM for regulatory enforcement**
  - **The BAM is one of FDA's major outreach to the world of food microbiology**
  - **BAM methods are used by developing countries for food exports to the US**

BAM 2018

- **BAM council**
  - **Chair: Karen Jinneman (ORA)**
  - **CFSAN: William Burkhardt, Peter Feng, Thomas Hammack, Julie Kase**
  - **ORA: Patrick Regan, Greg Gharst**
  - **CVM: Maureen Davidson, Beilei Ge**
  - **OFVM: Sunee Himathongkham**

BAM 2018

- **Activities**
  - **Monthly meetings**
  - **Chapter Revisions and Additions**
    - **New *Cyclospora* Chapter**
    - **Revised microbiological methods validation guidelines**
    - **Rewritten *Listeria monocytogenes* Chapter**
    - ***Salmonella* and *Shigella* updates**
    - **All methods will have a molecular component**

BAM 2018

- **How are methods selected for inclusion into the BAM?**
  - **There must be a need**
  - **There must be a significant improvement over current methodology in terms of sensitivity/specificity and/or time**
  - **Must be validated**
    - **Validation Study and Report must be approved by Microbiology Methods Validation Subcommittee**
  - **Must be approved by the BAM Council**
  - **Must be developed by ORA and/or CFSAN personnel**

FDA's Methods Validation Guidelines

The Science and Research Steering Committee (SRSC), of the Office of Foods and Veterinary Medicine (OFVM), approved guidance to be used for validation of microbiological and chemical methods.

Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods

http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf

Guidelines for the Validation of Chemical Methods for the FDA Foods Program

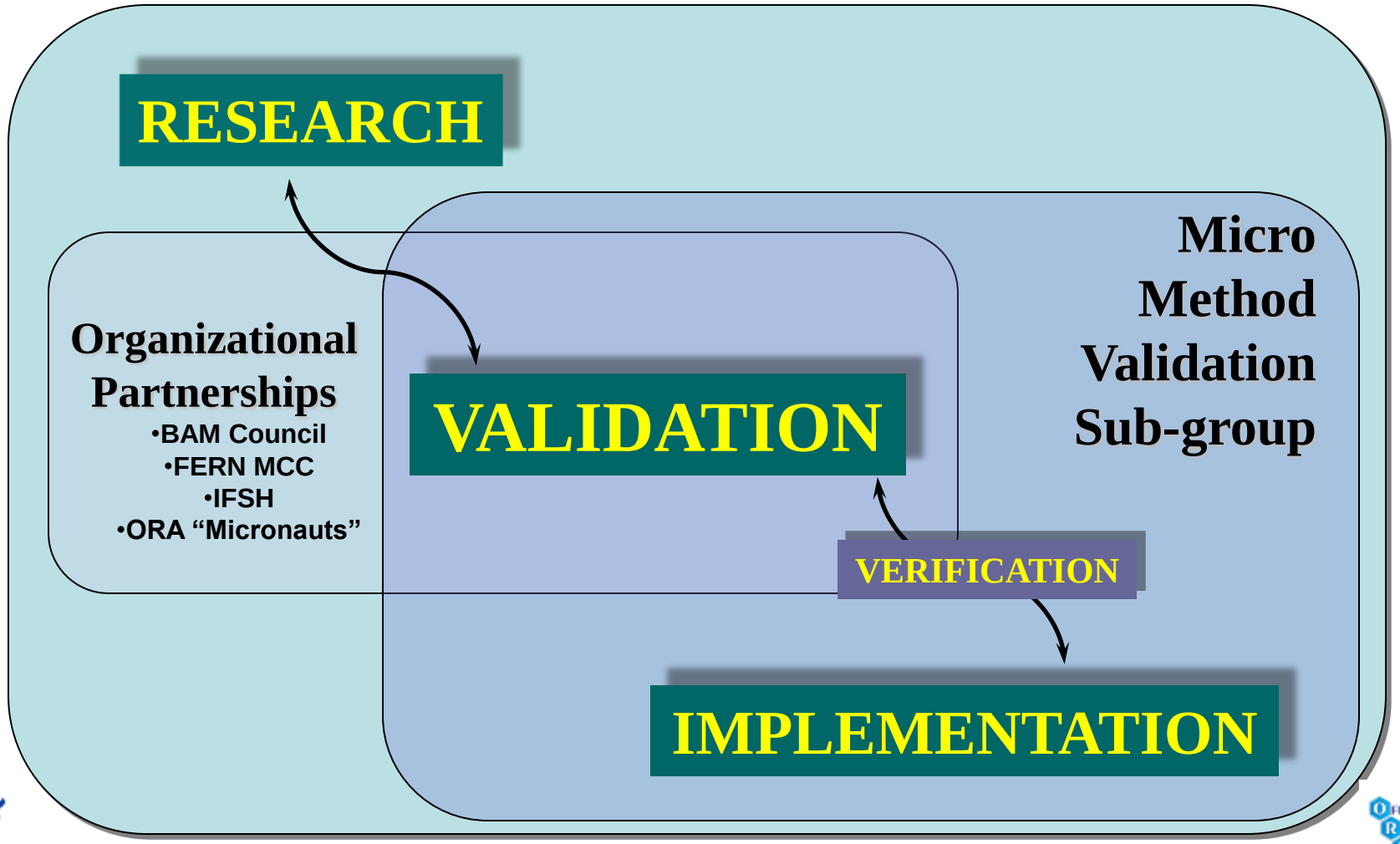
http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf

Scope

"These criteria apply to all FDA laboratories that develop and participate in the validation of analytical food methods for Agency-wide implementation in a regulatory capacity. This includes all research laboratories, and field labs where analytical methods may be developed or expanded for potential regulatory use. These documents will supersede all other intra-agency documents pertaining to food-related method validation criteria for microbial and chemical analytes. the SRSC will authorize the formation of a Methods Validation Subcommittee (MVS) to serve as the governing body for all method validation concerns."

The Office of Foods and Veterinary Medicine & the SRSC

"Roadmap for Microbiological Method Development and Validation"



The Method Validation Subcommittee (Microbiology)

ESTABLISHES validation needs and priorities in consultation with the SRSC-Micro Super-group, FDA *Bacteriological Analytical Manual* Council (BAM Council), FERN Method Coordinating Committee, ORA "micronauts" inter-center working groups and others as appropriate

ADOPTS procedures to govern all administrative processes needed for emergency and non-emergency method validation proposals and studies.

PROVIDES planning, guidance, oversight, and resources to participating laboratories during the method development and validation process; will be the responsible authority for recommendations, evaluations and final approval of all validation studies from planning through field implementation.

CONSULTS with other governmental, and independent (commercial, and international) validation bodies to harmonize validation standards where possible and practices

The Microbiology Methods Validation Subcommittee

BROAD REPRESENTATION from CFSAN, ORA, CVM, and NCTR with additional expertise from biostatisticians and QA/QC managers

CURRENT MICRO MMVS COMPOSITION:

OFVM: Sunee Himathongkham
ORA: Ian Joseph, Ken Yoshitomi, Terri McConnell, Zachary Miller, Jennifer Brzezinski
CFSAN: Thomas Hammack (Chair), William Burkhardt, Darcy Hanes, Steven Wang
CVM: Beilei Ge, Xin Li
NCTR: Ashraf Khan
FERN: Don Burr
NCFST (CFSAN Moffett): Ravinder Reddy

Method Validation is Required for…

1. **Submission of a new or original method, *OR,***

2. **Any significant modification of a method that may alter its performance specifications or changes to the fundamental science of an existing method. Categories include:**

   - **Substitutions of reagents/apparatus**
   - **Expansion of the scope of an existing method to include additional analytes.**
   - **Changes in intended use *i.e.* screening or confirmatory.**
   - **Platform extensions or significant parameter changes e.g. adaptation to another real-time PCR thermal cycler.**
   - **Matrix extensions.**
   - **Changes to time/temperature incubation periods, or enrichment media.**
   - **In cases where the sample preparation and/or the extraction procedure/analytical method is modified from the existing test procedure and protocol, *i.e the new method should demonstrate that the modifications do not adversely affect the precision and accuracy or bias of the data obtained.***
   - **Modification of a method's performance range *e.g.* specificity, sensitivity beyond previously validated levels.**

Levels of Validation

Two levels of performance are defined: emergency and non-emergency (SLV, Independent Lab, MLV). The hierarchy of scrutiny will provide general characteristics on the method's utility and insights for its intended use, the assessed risk, and the food-borne illness potential for an analyte-matrix pairing.

Not all methods will or should be validated to meet the requirements of a full collaborative study.

Table 1- General Guidelines for the Validation of Qualitative Detection Methods for Microbial Analytes

	Emergency	Non-Emergency Validation Processes		
Criteria	**Emergency Use**	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	‡TBD	50 (unless 50 aren't available)[b,c]	≠NA	≠NA
# of non-target organism (exclusivity) [a]	‡TBD	30 strains[d]	≠NA	≠NA
# of laboratories providing usable data	1	1	1	10
# of foods	1or more[e]	1or more[e]	1or more[e]	1or more[e]
# of analyte levels/food matrix	‡TBD	Two inoculated levels[f] and one uninoculated level	Two inoculated levels[f] and one uninoculated level	3 levels: One inoculated fractional level[f], one at 1 log higher[g] and one uninoculated level
Replicates per food at each level tested	‡TBD	20 for the fractional level (5 each for the uninoculated and high levels)	20 for the fractional level (5 each for the uninoculated and high levels)	8
Aging of inoculated samples prior to testing	No	Yes[h]	Yes[h]	Yes[h]
Addition of competitor strain[i]	Normal background flora	In 1 food at +1 log>analyte at fractional positive[f] analyte level	In 1 food at +1 log>analyte at fractional positive[f] analyte level	In 1 food at +1 log>analyte at fractional positive[f] analyte level
Reference Method Comparison Requirement[j]	‡TBD	Yes, if available	Yes, if available	Yes, if available

Table 2 - General Guidelines for the Validation of Qualitative Detection Methods for Microbial Analytes - Unique Isolation and/or Enrichment Challenges †

	Emergency	Non-Emergency Validation Processes		
Criteria	**Emergency Use**	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	Originating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)[a]	‡TBD	‡TBD	≠NA	≠NA
# of non-target organism (exclusivity)[a]	‡TBD	‡TBD	≠NA	≠NA
# of laboratories providing usable data[b]	1	1	1	5¥
# of foods	1 or more¥	1 or more¥	1 or more¥	1 or more¥
# of analyte levels/food matrix	‡TBD	One inoculated level[c] and one uninoculated level	One inoculated level[c] and one uninoculated level	3 levels: One inoculated level[c], one at 1 log higher[d] and one uninoculated level
Replicates per food at each level tested	‡TBD	3	3	8¥
Reference Method Comparison Requirement[e]	‡TBD	Yes, if available	Yes, if available	Yes, if available

Table 3- General Guidelines for the Validation of Identification Methods for Microbial Analytes

	Non-Emergency Validation Processes		
Criteria	**Single Laboratory Validation Study**	**Independent Laboratory Validation Study**	**Collaborative Validation Study**
Participating Laboratory	**Originating Laboratory**	**Collaborating Laboratory**	**Collaborating Laboratories**
# of target organism (inclusivity)[a]	≥50 (unless 50 aren't available)[b,c]	1[c]	12[c]
# of non-target organism (exclusivity) [a]	≥30 strains[b,c]	1[c]	12[c]
# of laboratories providing usable data	1	1	10
Replicates[d]	3	3	3
Reference Method Comparison Requirement	Yes, if available	Yes, if available	Yes, if available

Table 4- General Guidelines for the Validation of quantifiable Detection Methods for Microbial Analytes

	Non-Emergency Validation Processes		
Criteria	Single Laboratory Validation Study	Independent Laboratory Validation Study	Collaborative Validation Study
Participating Laboratory	Originating Laboratory	Collaborating Laboratory	Collaborating Laboratories
# of target organism (inclusivity)	50 (unless 50 aren't available)	NA≠	NA≠
# of non-target organism (exclusivity)	30 strains	NA≠	NA≠
# of laboratories providing usable data	1	1	10
# of foods	1 or more[a]	1 or more[a]	1 or more[a]
# of analyte levels/food matrix[f]	4 levels: Low medium and high inoculum levels[b] and one uninoculated level	4 levels: Low medium and high inoculum levels[b] and one uninoculated level	4 levels: Low medium and high inoculum levels[b] and one uninoculated level
Replicates per food at each level tested	5 replicates per level for a total of 20 replicates per method	5 replicates per level for a total of 20 replicates per method	Two test portions per level for a total of 8 test portions
Aging of inoculated samples prior to testing	Yes[c]	Yes[c]	Yes[c]
Addition of competitor strain[d]	In 1 food at +1 log>analyte at highest analyte level	In 1 food at +1 log>analyte at highest analyte level	In 1 food at +1 log>analyte at highest analyte level
Reference Method Comparison Requirement	Yes, if available	Yes, if available	Yes, if available
Confirmation of Test Portions	NA≠	NA≠	Yes, follow the reference method

BAM 2018

- **Needs**
  - **Improved culture methods**
  - **Molecular identification methods**
    - **Confirmatory**
    - **Subtyping/serotyping**
  - **Molecular detection methods**
    - **Screening**

What are the "Standard Methods" and What Makes Them So Special?

Standards and standardisation

An International Standard provides rules, guidelines or characteristics for activities or for their results, aimed at achieving the optimum degree of order in a given context. It can take many forms. Apart from product standards, other examples include : test methods, codes of practice, guideline standards and management systems standards.

Standardisation is the process to come to a standard.

Standardisation is based on consensus

ISO and CEN

- ISO: International Organisation for Standardisation

ISO is an independent, non-governmental international organization with a membership of 161 national standards bodies.

- CEN: European Committee for Standardization

CEN is a private international non-profit organization with in total 34 members (National Standardization Bodies)

Relation between ISO-CEN-National bodies

Global level (ISO)
- ISO-standards
- **Voluntary** at national level

European level (CEN)
- EN-standards
- **Obligatory** at European level

National level (e.g. NEN in Netherlands or ANSI in USA)
- NEN-standards or ANSI-standards
- National level needed for access to international level

Relation between ISO-CEN-National bodies

Global level (ISO)

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National level (e.g. NEN in Netherlands or ANSI in USA)

- NEN-standards or ANSI-standards
- National level needed for access to international level

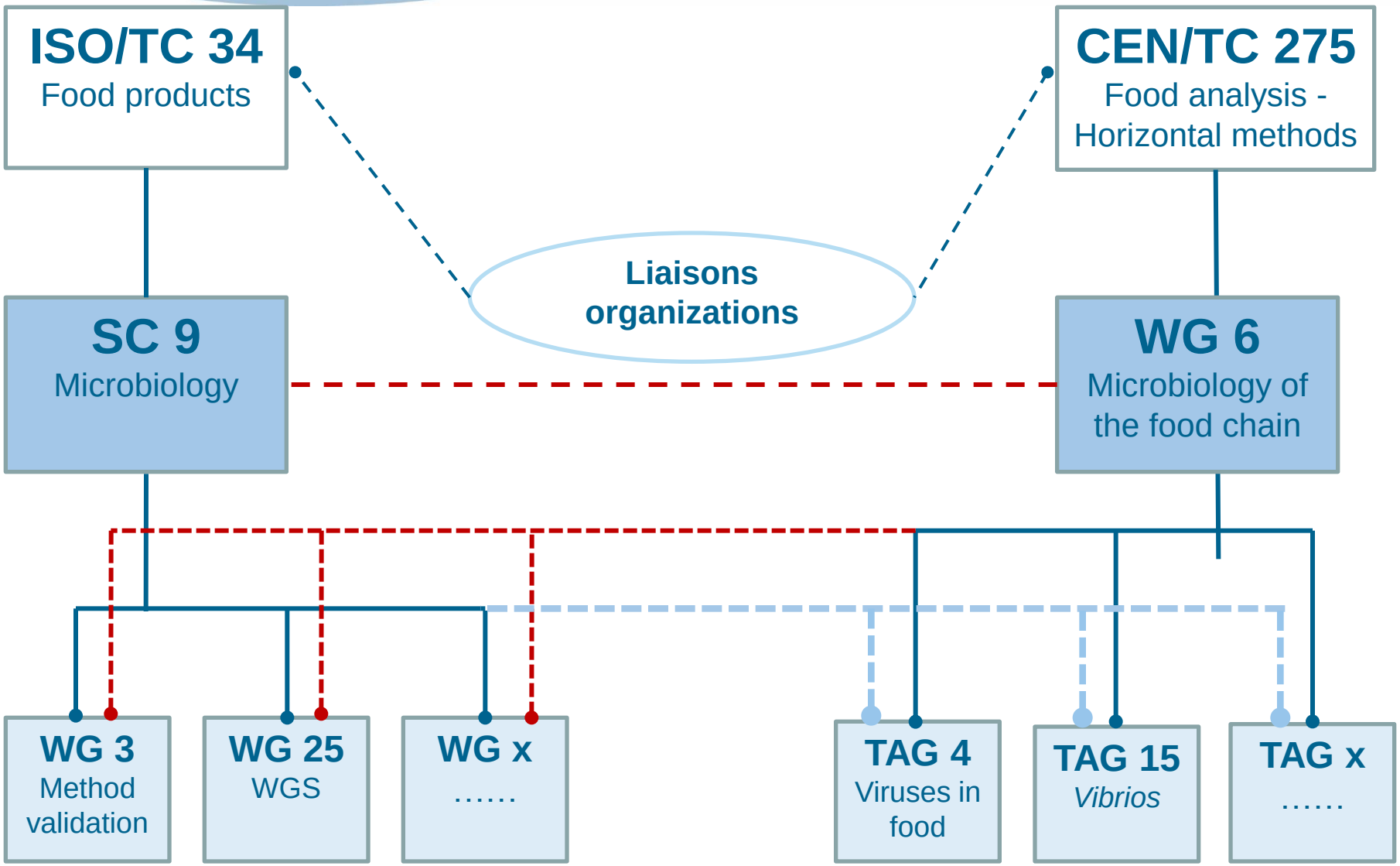
Nederlandse norm

NEN-EN-ISO 16140-2

(en)

Microbiologie van de voedselketen - Validatie van methoden - Deel 2: Protocol voor de validatie van alternatieve (eigendomsrechtelijke) methoden tegen een referentiemethode (ISO 16140-2:2016,IDT)

Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016,IDT)


ISO/TC 34
Food products
CEN/TC 275
Food analysis - Horizontal methods
Liaisons organizations
SC 9
Microbiology
WG 6
Microbiology of the food chain
WG 3
Method validation
WG 25
WGS
WG x
……
TAG 4
Viruses in food
TAG 15
Vibrios
TAG x
……

ISO TC 34/ SC 9

- Responsable for ca 80 standards
- Working area Food Microbiology including bacteria, virusses, yeast/moulds, parasites, toxins and metabolites
- Work done in total 24 Working Groups
- Stages during development:

Development of standards (ISO/CEN)

Under Vienna Agreement

Stages	CEN	ISO
0) Preliminary stage **[optional]**	Preliminary Work Item **(PWI)**	Preliminary Work Item **(PWI)**
1) Proposal stage **[mandatory]**	Activation of New Work Item **(NWI)**	New Work Item Proposal **(NP)** *[enquiry within ISO/TC 34/SC 9 – **national committees can vote and comment**]*
2) Preparatory stage **[optional]**	Working Draft **(WD)**	Working Draft **(WD)** *[commenting possibility in WGs]*
3) Committee stage **[optional]**		Committee Draft **(CD)** *[**internal enquiry** within ISO/TC 34/SC 9 – **national committees can vote and comment**]*
4) Enquiry stage **[mandatory]**	Draft European Standard **(prEN)**	Draft International Standard **(DIS)** *[**public enquiry** – **national committees can vote and comment**]*
5) Approval stage **[optional]**	Draft European Standard for Formal Vote **(FprEN)**	Final Draft International Standard **(FDIS)** *[**national committees can vote and comment** (editorial)]*
6) Publication stage **[mandatory]**	**EN**	**ISO**

ISO TC 34/ SC 9

- Different types of standards:
  - Analytical methods (pathogenes and non-pathogens)
  - Sampling (e.g. surfaces or primary production samples)
  - General standards ….
- Standards are based on freely available techniques (non-proprietary)
- Standards periodically reviewed (every 5 years)

Analytcial method examples:

- *Salmonella*: ISO 6579-1 to 3 (detection, MPN, serotyping)
- *E.coli* O:157: ISO 16654
- STEC: ISO-TS 13136
- *Listeria spp.* and *L. monocytogenes*: ISO 11290-1 and 2 (detection and quantification)
- Norovirus en HEP-A: ISO 15216-1 and 2 (detection and quantification)
- Staph. enterotoxin: ISO 19020 (immuno-enzymatic methods)
- *B.cereus* emetic toxin: ISO 18465 (LC-MS method)
- ……

General standards examples

- ISO 7218: General rules for microbiological examinations
- ISO 6887-1 to 6: pre-treatment of samples
- ISO 20976-1: Challengetest to study the growth potential and the maximum growth rate (expected in 2019)
- ISO 19036: Guidelines for estimating measurement uncertainty
- ISO 17468: Technical requirements and guidance on establishment or revision of a standardized reference method
- ISO 16140-1 to 6: standards on validation and verification

ISO 16140 series

- Part 1: terminology (2016)
- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (2016)
- Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory (expected in 2019)
- Part 4: Protocol for single-laboratory (in house) method validation (expected in 2019)
- Part 5: Protocol for factorial interlaboratory validation of non-proprietary methods (expected in 2019)
- Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures (expected in 2019)

European legislation and methods

- European Directive 2073/2005: Microbiological criteria.
- Lays down the microbiological criteria for certain micro-organisms and the implementing rules to be complied with by food business operators.
- Example of those criteria

Chapter 1. Food safety criteria

Food category	Micro-organisms/their toxins, metabolites	Sampling-plan (1)		Limits (2)		Analytical reference method (3)	Stage where the criterion applies
		n	c	m	M		
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes (4)	*Listeria monocytogenes*	10	0	Absence in 25 g		EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2. Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g (5)		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
		5	0	Absence in 25 g (7)		EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3. Ready-to-eat foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (4) (8)	*Listeria monocytogenes*	5	0	100 cfu/g		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
1.4. Minced meat and meat preparations intended to be eaten raw	*Salmonella*	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf-life
1.5. Minced meat and meat preparations made from poultry meat intended to be eaten cooked	*Salmonella*	5	0	From 1.1.2006 Absence in 10 g From 1.1.2010 Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf-life

Chapter 1. Food safety criteria

Food category	Micro-organisms/their toxins, metabolites	Sampling-plan (1)		Limits (2)		Analytical reference method (3)	Stage where the criterion applies
		n	c	m	M		
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes (4)	*Listeria monocytogenes*	10	0	Absence in 25 g		EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2. Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g (5)		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
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1.3. Ready-to-eat foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (4) (8)	*Listeria monocytogenes*	5	0	100 cfu/g		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
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European legislation and methods

- For the criteria EN-ISO methods are mentioned as the reference method.
- Alle methods referred to in legislation are validated.
- In addition alternative methods can be used but under certain conditions (article 5)

Article 5 2073/2005

Proprietary methods may be used as alternative analytical methods if **validated against the specific reference method provided in Annex I** in accordance with the protocol set out in standard **EN ISO 16140-2** or other internationally accepted equivalent protocols, as described in the third subparagraph, and **certified by an independent organisation**, requiring an **evaluation of the manufacturer's production process assurance**. The certified proprietary method shall have a **certificate periodically renewed**, which includes a summary of and/or a reference to the validation results of the method and a statement on the quality management of the method's production process.

Article 5 2073/2005

In practice those method are mostly validated by Microval and NF-validation (AFNOR).

AOAC validated methods not directly suited mainly because of reference method used.

In addition difference between PTM and OMA methods.

Questions?