Need: Validated Food Microbiology Methods

It was recognized >30+ years ago, that proprietary methods:
• Generally cheaper
• Provide faster results versus traditional culture methods
• Often easier (less technical skill needed)

Acceptance of these methods by regulatory authorities?

Central European Norm (CEN) Eureka project started (now called MicroVal):
• Developed technical rules for validation
• Technical rules were transformed into a standardised (ISO) protocol
  • ISO 16140: Protocol for the validation of alternative methods
ISO 16140 and European legislation

EU Directive 2073/2005: Microbiological criteria for food stuffs

•Legislated the methods to be used per food stuff
  • Within the European Union
  • For Exporting TO the European Union

• These method should be either:
  • ISO methods
  • CEN methods

• OR – a proprietary method that meets these criteria:
  • Validated following ISO 16140
  • Compared to the Reference method
  • Certified by a third party
ISO 16140 within European Commission Regulations

• European “Commission Regulation No. 2073/2005 on microbiological criteria for foodstuffs”:

*COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs*

• Article 5 (Section 5) says:

*Article 5*

Specific rules for testing and sampling

The use of alternative analytical methods is acceptable when the methods are validated against the reference method in Annex I and if a proprietary method, certified by a third party in accordance with the protocol set out in EN/ISO standard 16140 or other internationally accepted similar protocols, is used.
Third Party Certification Schemes

that use ISO 16140:

- **AFNOR**: French National Organization for Standardization
- **MicroVal**: European certification body for microbiology methods
- **NordVal**: Nordic certification body: Denmark, Finland, Iceland, Norway, Sweden
Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods

Published in 2003

• ISO documents are reviewed every 5 years
• Amendment added in 2011
• Prepare for update to the standard
• Broader look at validation needs
**ISO 16140 Suite of Standards & Impact**

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ISO 16140-1 : 2016
Microbiology of the Food Chain – Method Validation – Part 1: Vocabulary

• Provides definitions for the terms used in the entire ISO 16140 Series
• 20 pages long
• 3 Clauses:
  1. Scope
  2. Terms and Definitions (83)
  3. Bibliography
Microbiology of the Food Chain – Method Validation – Part 2:
Protocol for the validation of alternative (proprietary) methods
against a reference method

Provides a protocol for the validation of proprietary methods
• Compared to the corresponding reference method
• Applicable to qualitative and quantitative methods
• Succeeds the first version of ISO 16140 (ISO 16140:2003).

Part 2 consists of two steps:
• Methods comparison study
• Inter-laboratory study

This STANDARD is:
• HARMONIZED with AOAC INTERNATIONAL Method Validation Guidelines (2012)
• Used to conduct method validation through a Certification Body
ISO/DIS 16140-3: Microbiology of the Food Chain – Method Validation – Part 3: *Protocol for the verification of reference and validated alternative methods implemented in a single laboratory*

Proposed as a 2 step procedure:

1. Verify using ONE matrix used in the validation study (ISO 16140-2)
2. Verify categories tested in your lab
   - For “Broad Range of Foods” = test (food) items from a minimum of 5 categories

*ISO 16140-2 lists 18 categories:*

- 15 food
- Animal feed
- Environmental
- Primary Production
ISO/DIS 16140-4
Microbiology of the Food Chain – Method Validation Part 4:
Protocol for single laboratory (in-house) validation

Addresses method validation within a single laboratory:
• Results are only valid in the laboratory which conducted the study
• Method verification (Part 3) is not required

Validation can be conducted using:
• Conventional method validation design (Part 2)
• Factorial method validation design (Part 5)
ISO/DIS 16140-5: Microbiology of the Food Chain – Method Verification – Part 5: Protocol for factorial inter-laboratory validation of non-proprietary methods

Method validation requires Inter-laboratory testing:
  • Challenging to find 8 (quantitative) and 10 (qualitative) labs to participate

ISO 16140-5 provides a protocol that:
  • Reduces required labs to 4-9, using factorial design to increase efficiency
  • Applies only to methods that have been fully specified and optimized, because:
    • Several factors are altered simultaneously (technician, culture medium)
    • Method is use in a range of different factor setting (time, temperature)
  • Can only be used for NON-proprietary methods
ISO/DIS 16140-6:
Microbiology of the Food Chain – Method Verification – Part 6:
Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing

Somewhat different from other parts of ISO 16140 series:
• Specific to where only the CONFIRMATION procedure of a method is validated
• Confirmation advances a suspected (presumptive) result to a confirmed result
• Typing of pure strains (e.g. serotyping of Salmonella) is included

Validation includes comparison to the reference confirmation procedure

Intended for “full” validation of an alternative (proprietary) method through confirmation and typing = alternative confirmation method
ISO 16140 Series

ISO has recognized several ISO 16140 documents as “high profile” because they believe the global food industry has a great need for these documents:

- **ISO 16140-2**  Method *Validation* - Published August 2016
- **ISO 16140-3**  Method *Verification* – Expected publication 2019

Decision to gather input from USER LABORATORIES, vs just WG3 Experts
Acceptance Criteria defined BEFORE starting

• Responses from > 30 global laboratories
  • Various lab sizes
  • Global regions (including Africa/Middle East if possible)
  • Industry, Contract, Government

• ALL responses to the questionnaire rated ≥ 3 on a 1-5 scale

• 75% of the user laboratories are able to follow and understand ISO/CD 16140-3, and (for those that attempted) are able to conduct a verification
User Laboratory Response

52 of 60 labs responded = 80% response rate!
User Laboratory Participation

**Regions**
- Asia Pacific: 31%
- Europe: 21%
- Latin America: 4%
- Middle East/Africa: 8%
- North America: 36%

**Countries**
- Australia
- Belgium
- Brazil
- Canada
- Chile
- China
- Finland
- France
- Germany
- India
- Iran
- Italy
- Japan
- Malaysia
- Singapore
- South Africa
- Spain
- Switzerland
- Thailand
- The Netherlands
- United Kingdom
- United States
User Laboratory Evaluation: \textit{Text Comprehension}

Acceptance criteria: $75\% \geq 3$ (neutral)
User Laboratory Evaluation: *Text Comprehension*

**Qualitative Methods**

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Acceptance criteria: $75\% \geq 3$ (neutral)
User Laboratory Evaluation: *Text Comprehension*

Quantitative Methods

Acceptance criteria: $75\% \geq 3$ (neutral)
User Laboratory Evaluation: Practice

Verification on site

Acceptance criteria: 75% ≥ 3 (neutral)
Next Steps: ISO 16140 parts 3-6

Part 3:
- Round 1 (Q1 2017):
  - 60 pages of comments from global WG3 members
  - >500 comments from the User Lab Evaluations
- Round 2 (Q3 2017):
  - ~150 comments from global WG3 members
  - Create a “transition document” - to help labs implement ISO 16140-3

Parts 3-6:
- Submitted for Draft International Standard (DIS) review by SC9, early 2018
- Respond to SC9 comments → Final Draft International Standard (FDIS)

Publication of all 4 expected in 2019
Gracias

Thank you!