3M Science. Applied to Life.™

ISO 16140 Series:
Standards for Microbial Method Comparison

INOFOOD 24 October 2017
Santiago, Chile

DeAnn Benesh, 3M Food Safety

- President, AOAC INTERNATIONAL
- Co-Chair ISO 16140-3

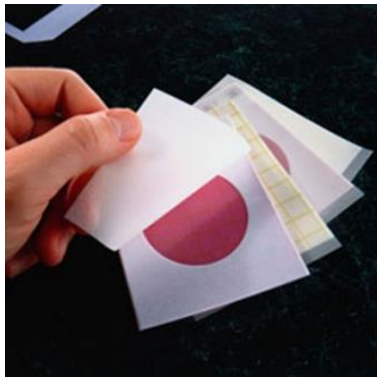
AOAC INTERNATIONAL

ISO International Organization for Standardization

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

I.S. EN ISO 16140:2003

INTERNATIONAL STANDARD

ISO 16140

First edition
2003-05-01

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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1. Terminology	Parts 2-6
2. Validation of (alternative) Methods	Certification
3. Methods Verification	Accreditation
4. In-house Single Laboratory Method Validation	Accreditation
5. Factorial Multi-laboratory Method Validation	Accreditation
6. Validation for Confirmation Methods	Certification

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

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

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

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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

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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)

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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*

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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

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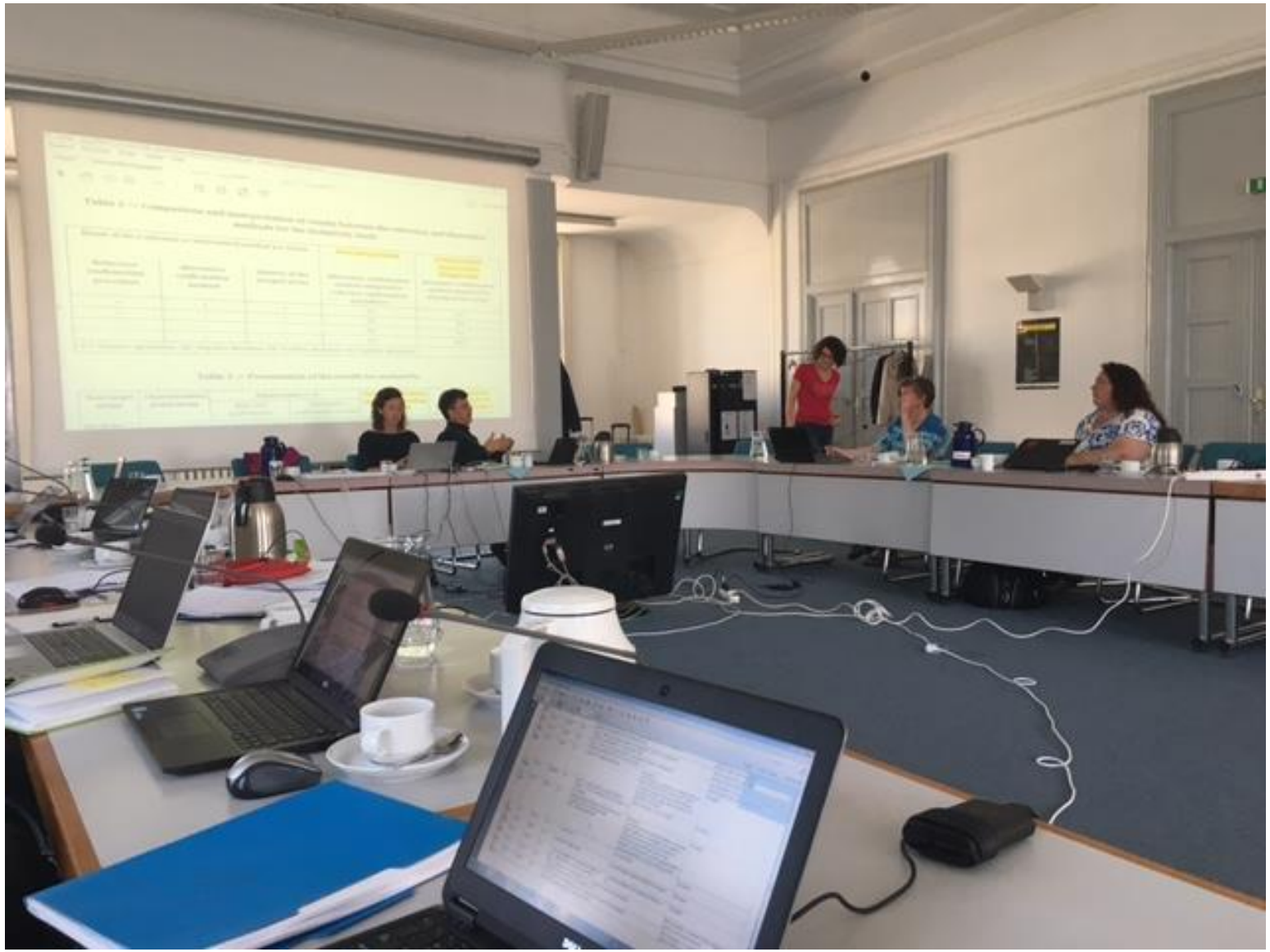
ISO TC34 (food)/SC9 (microbiology)/WG3 (methods) Meeting



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Utrecht, Netherlands
20-22 Sept 2017

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**

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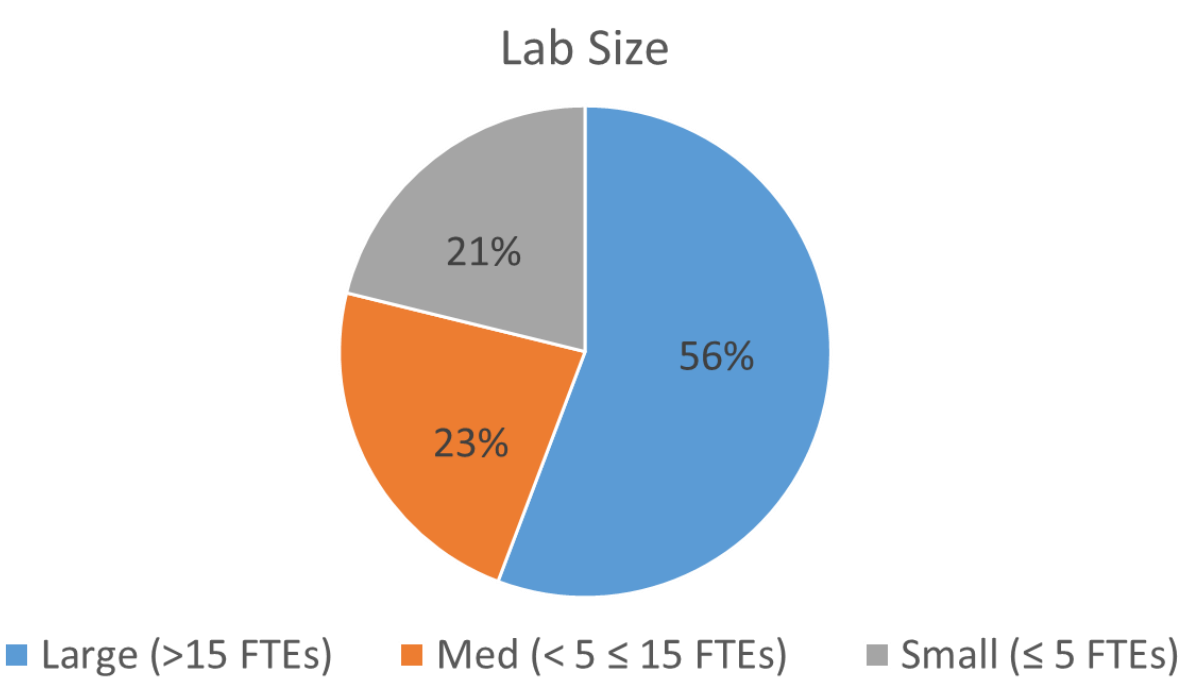
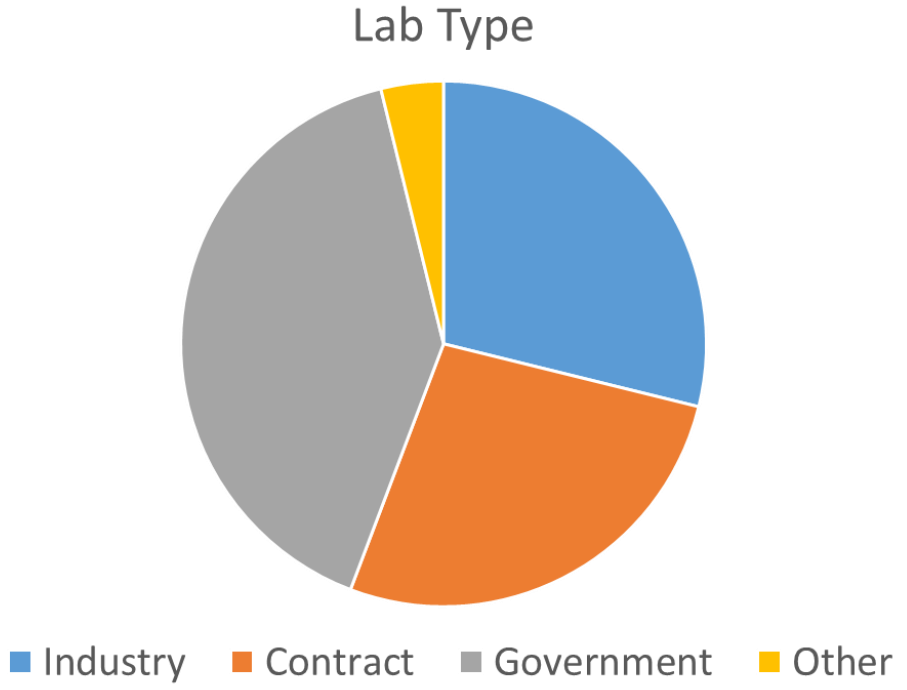
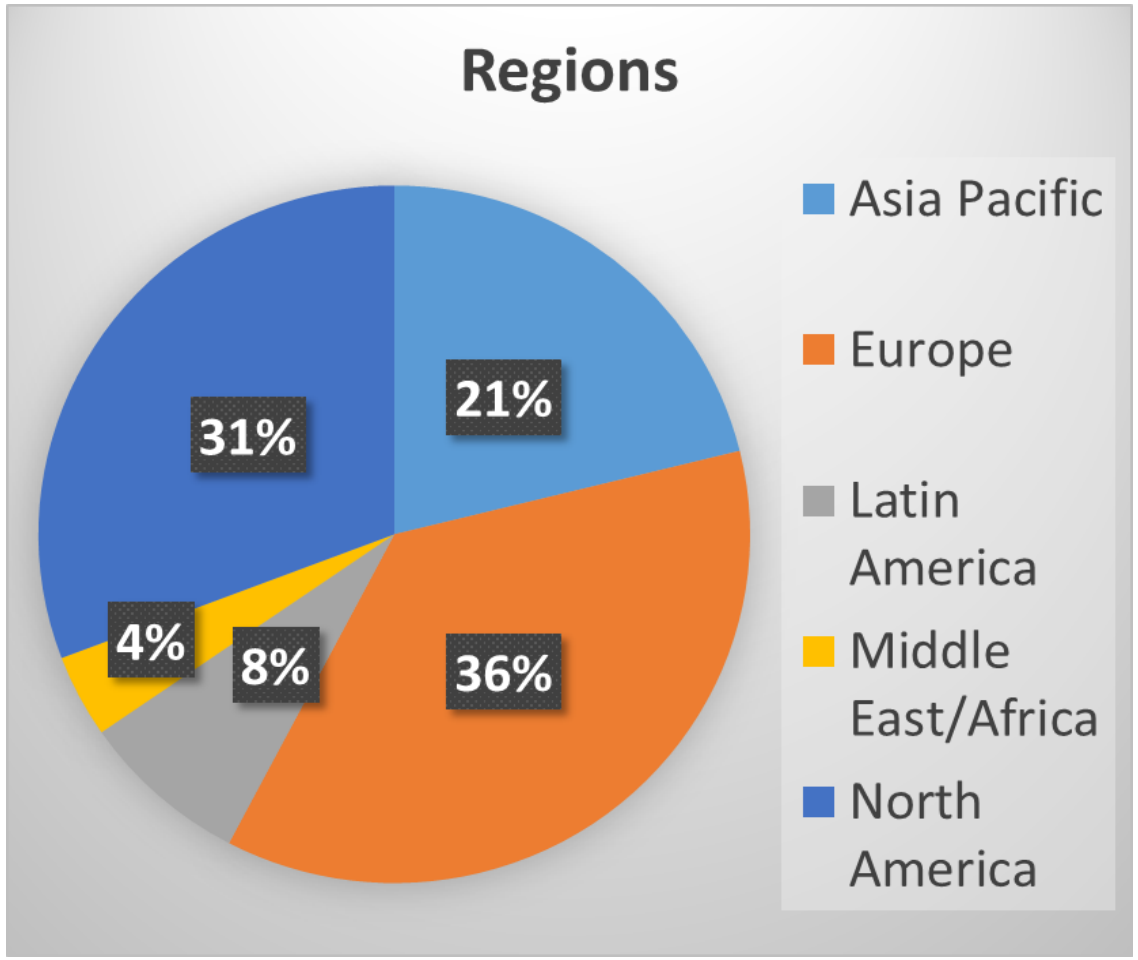
User Laboratory Response

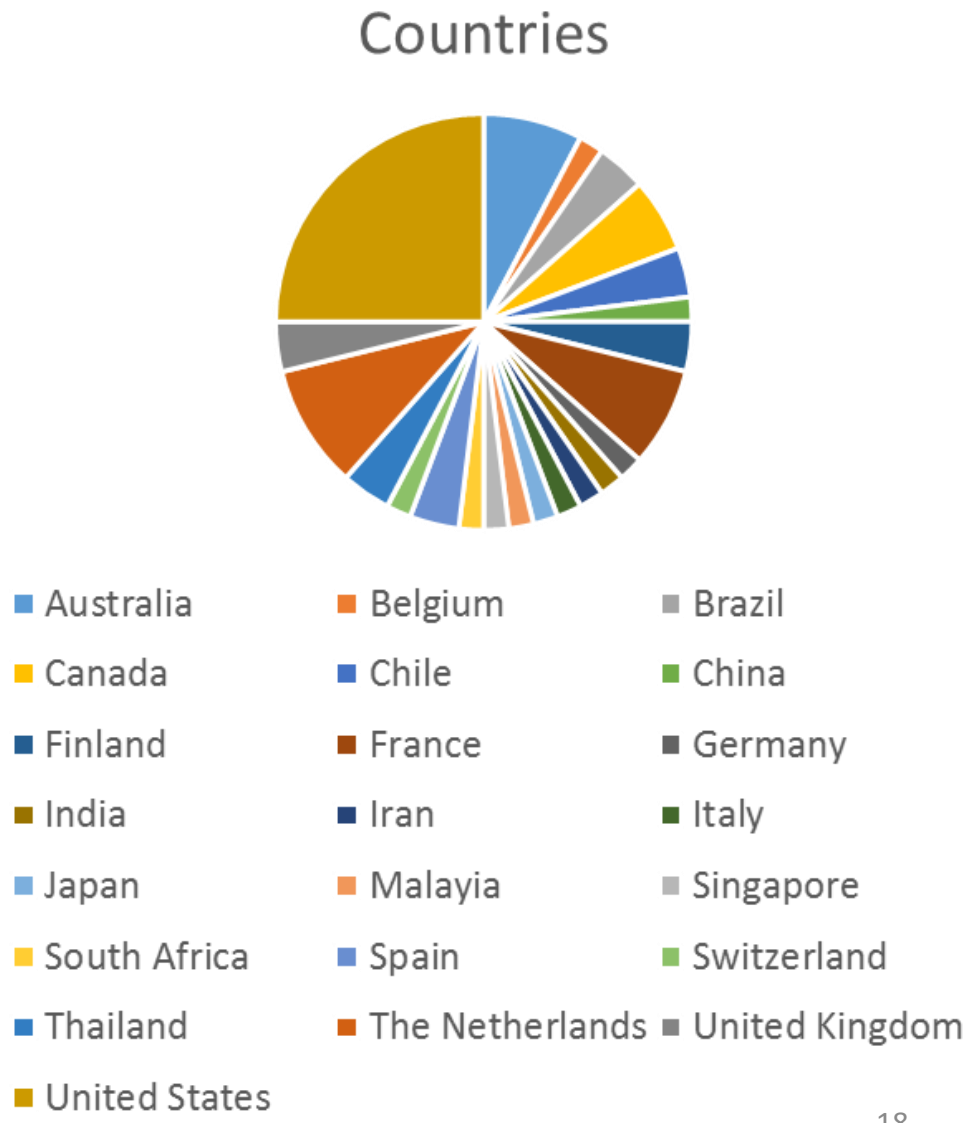
52 of 60 labs responded = 80% response rate!



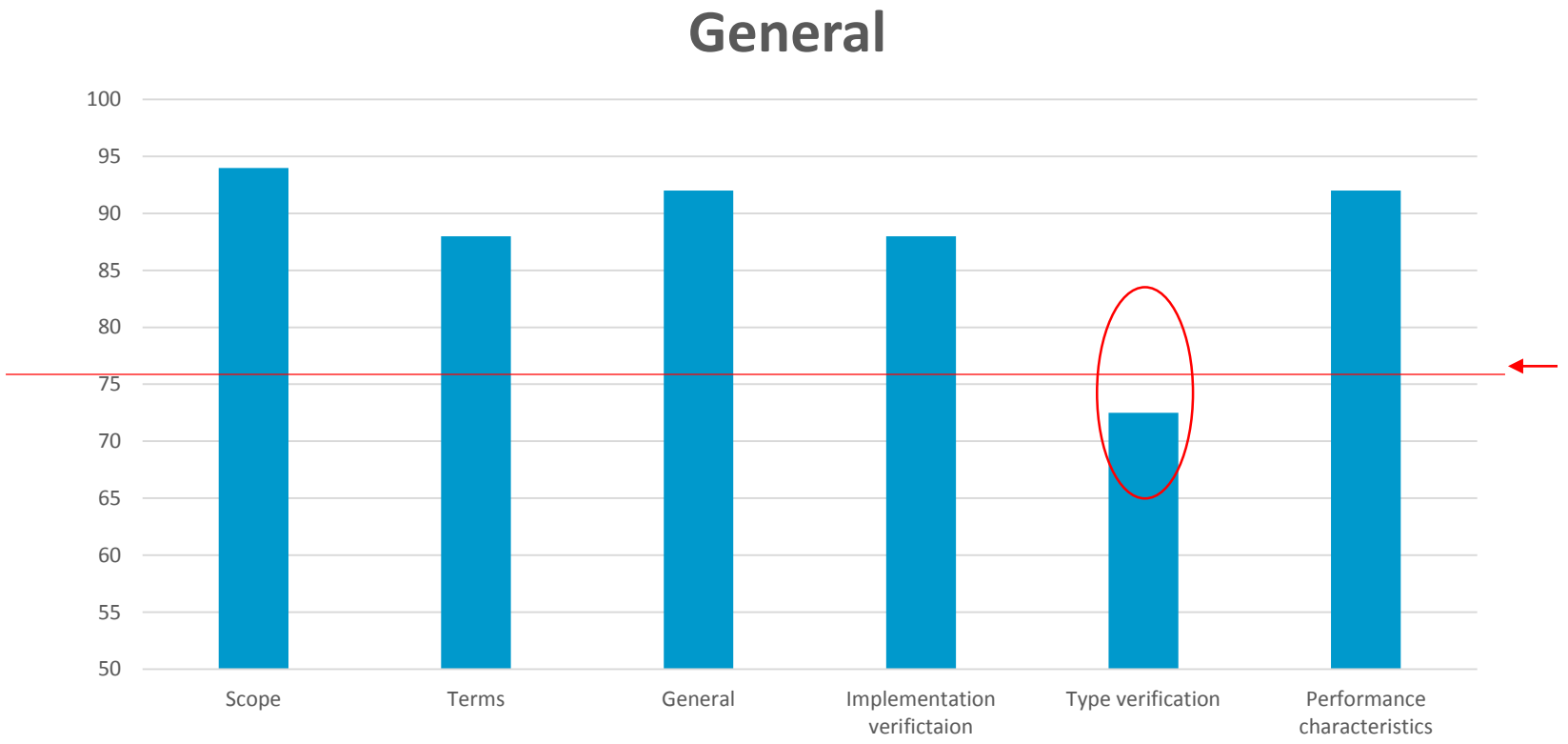


User Laboratory Participation





User Laboratory Evaluation: *Text Comprehension*



Acceptance criteria:
75% ≥ 3 (neutral)

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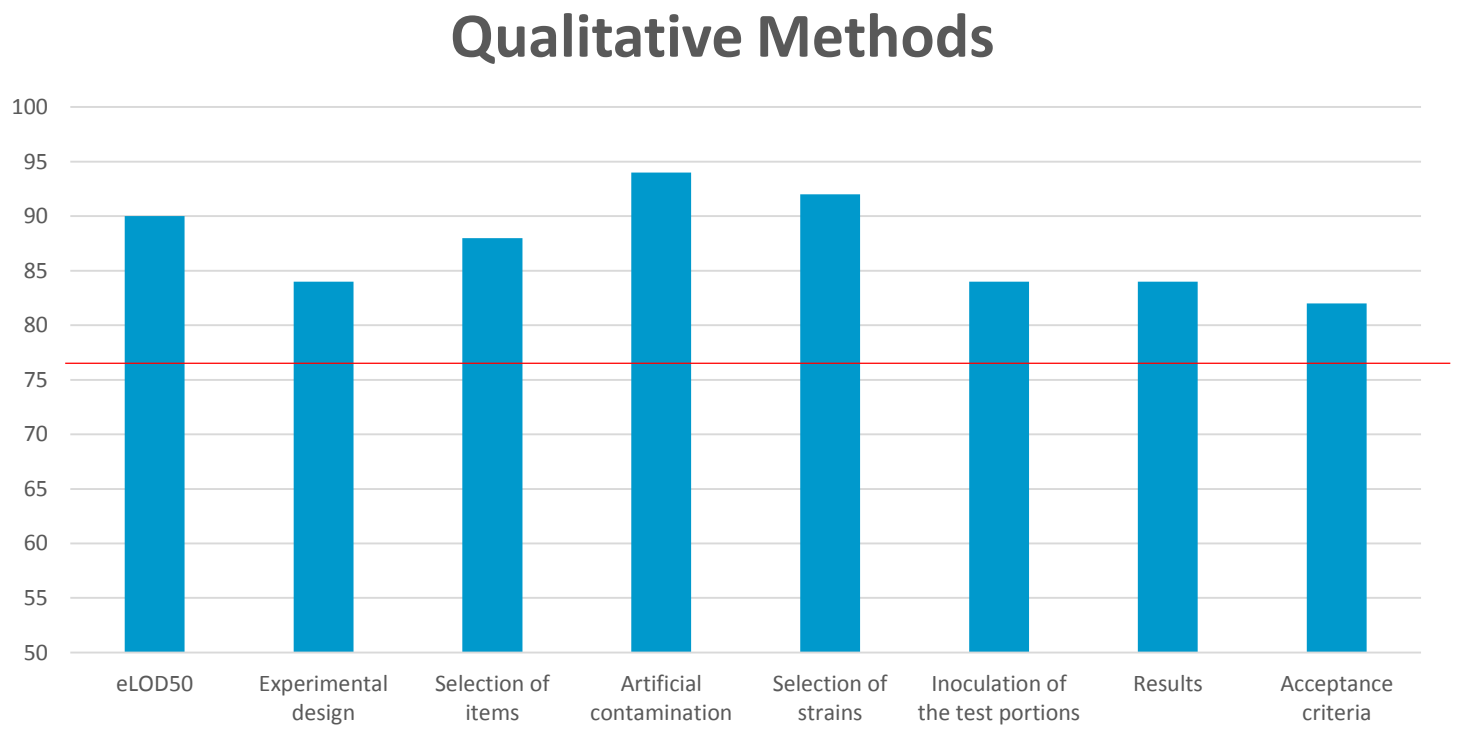
User Laboratory Evaluation: *Text Comprehension*



Acceptance criteria:
75% ≥ 3 (neutral)

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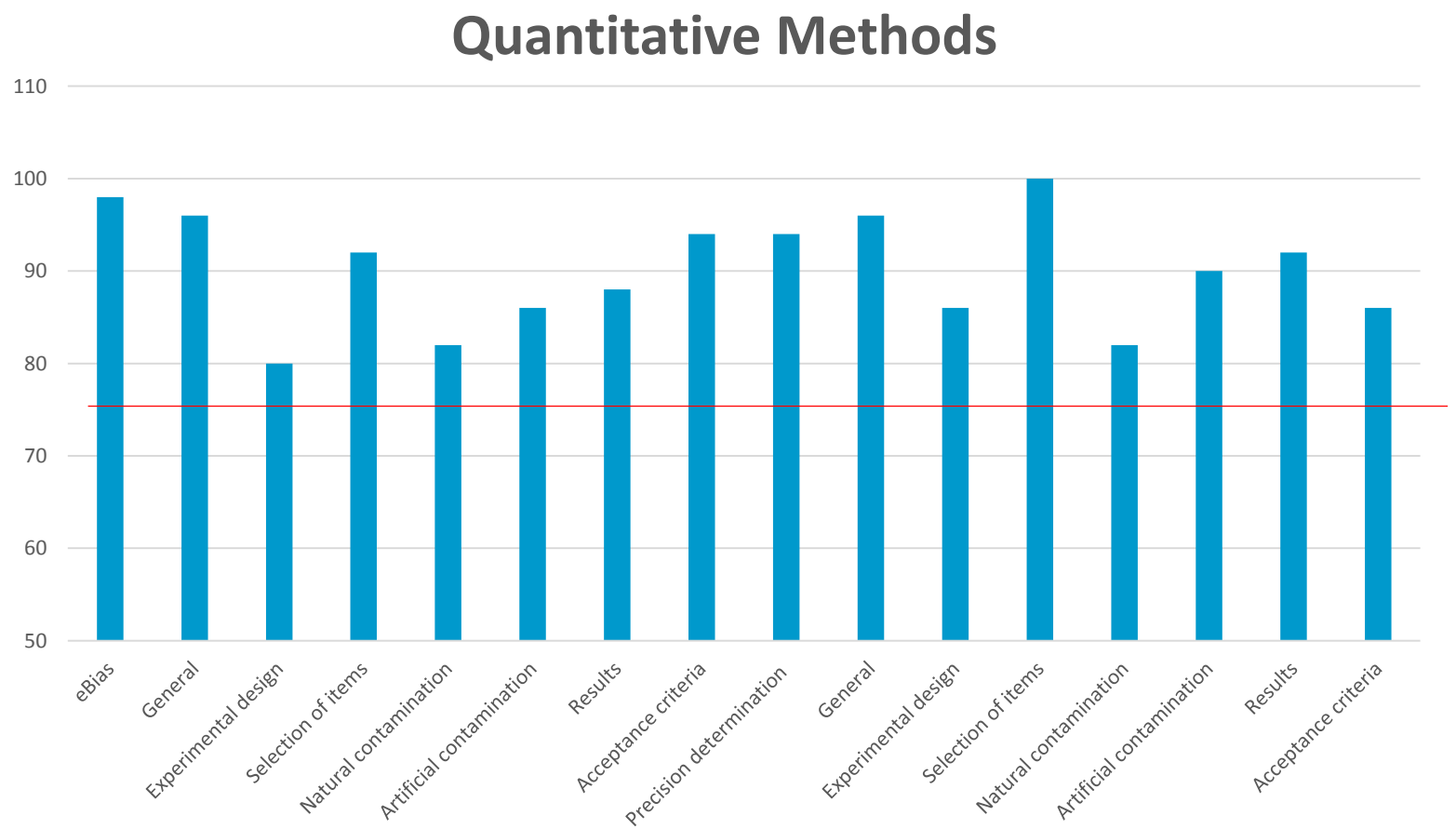
User Laboratory Evaluation: *Text Comprehension*



Acceptance criteria:
75% ≥ 3 (neutral)

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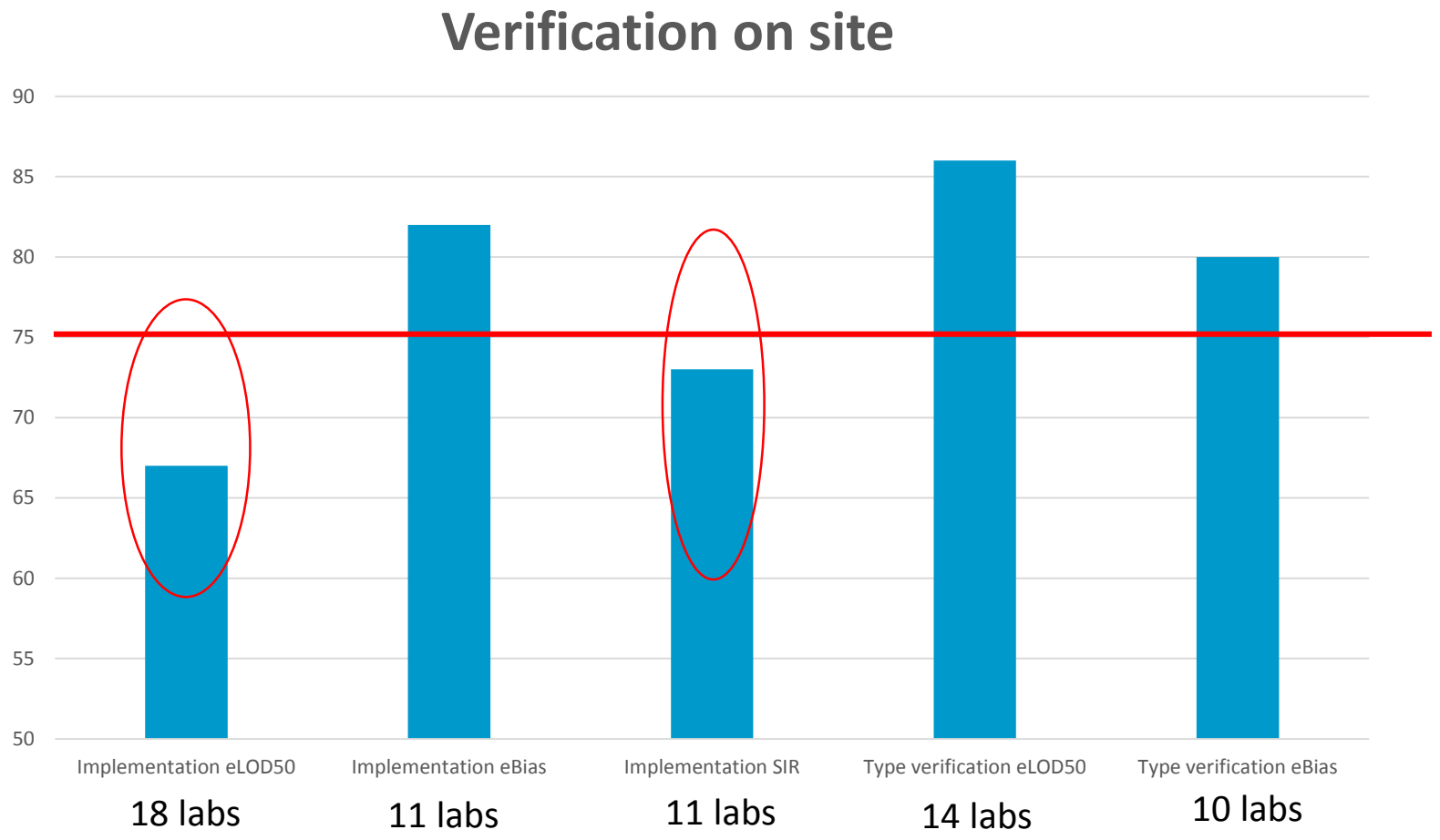
User Laboratory Evaluation: *Practice*



Acceptance criteria:
75% ≥ 3 (neutral)

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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!

3M