# Validation of Biological Testing Methods

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There are various biological testing methods in vogue and are associated with varied amount of complexities based up on their application.

- Screening and Confirmation methods
- Instrumental: Hybrid Methodology, alternate platforms
- Regulatory approved methods
- Elementary vs Technologically perplexed systems

Microbiological Testing Methods are prone to challenges such as logistical complexities in sampling; heterogeneous distribution of contaminant flora; high level of background flora; interfering ingredients; stress-Injury; viable but Non-culturable State; and high dependence on culture-based methods. The 50% of global testing in microbiology is still carried out in traditional media.

- Complexities of Target organisms
  - Bacteria
  - Fungi
  - Virus
  - Parasites

- Complexities of Methods
  - ISO
  - AOAC
  - USFDA-BAM
  - APHA
  - Health Canada
  - Country-specific NSBs

**Why do we need Validation/Verification of Rapid Food Testing Kits?**

- New tools must perform equal to or better than standard culture based methods
- Rapid tools perform better only in some food matrices

**Guidelines and Standards for Validation/Verification of Microbiological Rapid Food Testing Kits**

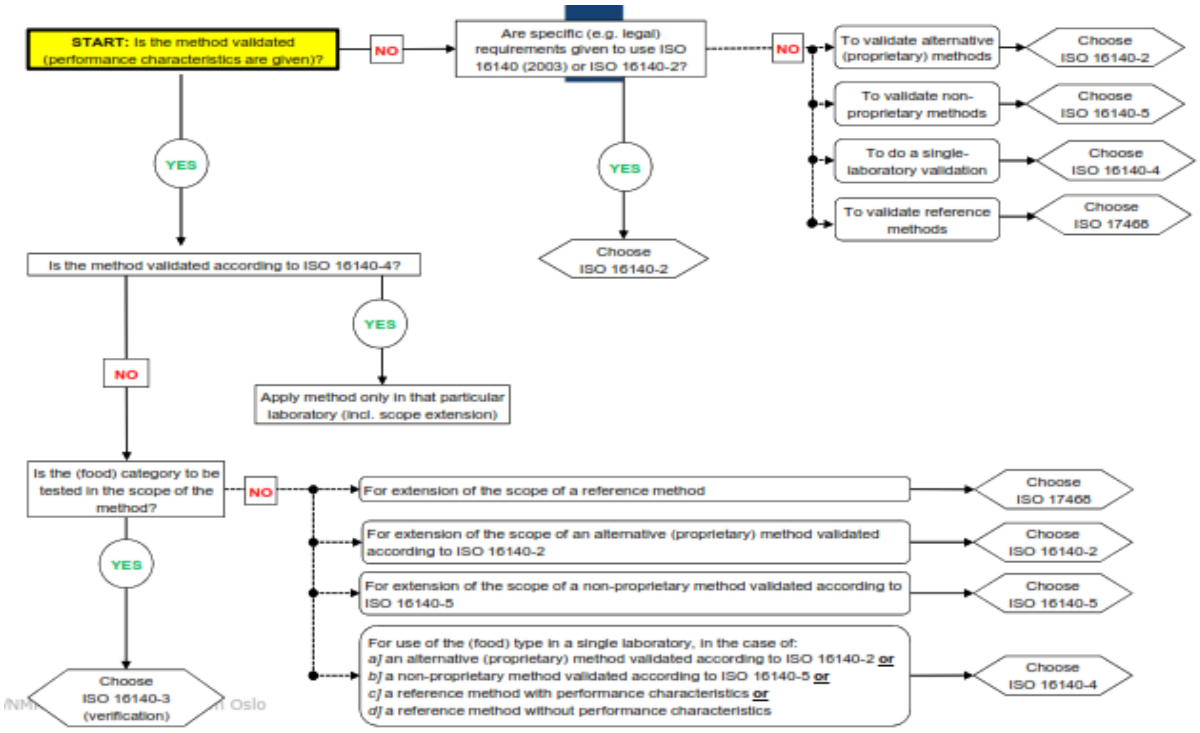
- Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, Edition 3.0, U.S. Food and Drug Administration Foods Program, October 2019

- AOAC® Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces
- ISO 16140 series of standards

**ISO Standards on Microbiology Method Validation**

- ISO 16140-1:2016 Microbiology of the food chain — Method validation — Part 1: Vocabulary
- 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
- ISO 16140-3: 2021 Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory
- ISO 16140-4:2020 Microbiology of the food chain — Method validation — Part 4: Protocol for method validation in a single laboratory
- ISO 16140-5:2020 Microbiology of the food chain — Method validation — Part 5: Protocol for factorial interlaboratory validation for non-proprietary methods
- ISO 16140-6:2019 Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures
- ISO 17468:2016 Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method

**Selection of appropriate ISO standard for validation**




**Validation of Microbiological Testing Methods as per ISO 16140-2**

During validation comparison is made between a reference method and an alternative protocol. Both for Qualitative and Quantitative methods can be validated using this standard. This comprises of two phases:

Phase I: method comparison study: using diverse food matrices

Phase II: Interlaboratory study: using single food matrix (reproducibility)

**Qualitative Method Comparison Study**

- Paired/Unpaired study
- Sensitivity study
  - 5 food categories; 60 samples
- RLOD study
  - 1 matrix per category, 20 samples per matrix
- Inclusivity/exclusivity study
  - Inclusivity: 50 target cultures (100 for Salmonella)
  - Exclusivity: 30 non-target cultures

**Quantitative Method Comparison Study**

- Relative Trueness Study
  - 5 food categories; 15 samples/category
- Accuracy profile study
  - 5 food categories; 6 samples/category (2low, 2 medium, 2 high)
- Limit of quantification study
  - Used where indirect detection (fluorescence, turbidity); 10 blank
- Inclusivity/exclusivity study
  - Not required for TPC/Y&M count
  - Inclusivity: 50 /Exclusivity:30

**Performance of Interlaboratory Study**

- 10 collaborators; 10 valid data sets
- Three different levels of contamination
- Simulate sample stabilization/stress
- At least 8 blind replicates
- Calculate specificity, sensitivity, relative trueness, false positive ratio
- Interpret with respect to specified acceptability limit