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Choosing a Method Validation Procedure

Many important decisions are made on the basis of analytical results. Getting the right result is therefore essential. Laboratories use a variety of approaches to help ensure that they get the right result. One of these is method validation.

Users of the analytical results may therefore require that a laboratory uses validated methods - either asking for this directly or by requiring the laboratory to be accredited against a standard which requires method validation. So, what exactly is validation and what types of validation are available to laboratory managers?

Validation is confirmation that the method meets the requirements for a specific intended use - that is, that the method is *fit for purpose*.

This document focuses on method validation - demonstration that the method is fit for purpose when performed correctly. Many "standard" methods have already been validated and the laboratory may not need to do further method validation, only laboratory assessment – that is, demonstration that a laboratory performs a method correctly. Laboratory assessment is a much wider issue, which would take in aspects such as accreditation (i.e. formal recognition that laboratory or person is competent to carry out specific tasks) and proficiency testing (inter-laboratory trials through which laboratories can judge their performance) – and is beyond the scope of this short paper.

Method validation inevitably involves some specific, technical language (i.e. jargon) and statistics. There is therefore a glossary at the end of this document.

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Types of method validation procedures

There are confusingly many 'standard' procedures indicating how methods should be validated. Two very influential organizations, each publishing a range of procedures, are:

- ISO - a worldwide federation of national standards bodies.
- AOAC - a globally recognized, independent, third party, not-for-profit association and voluntary 'consensus standards' developing organization.

Procedures differ in many ways including:

- Scope:
  - Method type: quantitative or qualitative analytical methods
  - Analysis type: such as chemistry, microbiology, drug development, and agriculture
  - Material: water, foods
- Procedure characteristics
  - Number of laboratories involved
  - Characteristics assessed; e.g. precision, trueness or bias

To help you choose an appropriate procedure, or anticipate which procedures you might be expected to follow, this document briefly describes some procedures published by some of the authoritative bodies. The descriptions are not intended to be complete – you will still need to read the actual procedures. Also, the list is not exhaustive – there are many procedures not covered here. Nevertheless, this document should help you avoid having to consult many procedures which are not appropriate to your circumstances.

The numbered sections below briefly describe each validation procedure. So that you don't have to read all the descriptions, Table 1 groups them into broad categories by nature of test (chemistry or microbiology), nature of result (Qualitative or Quantitative), and number of laboratories involved in the validation procedure (one or several).

For example, for a single laboratory validation of a quantitative microbiology method, procedures other than 1.1, 1.3, 1.5, 2.2, 3, 4 and 6 are not appropriate. Reading those seven descriptions should help you judge which might meet your needs, and for which you need to read the complete procedure documents to make a final decision.

Table 1: Categorisation of procedures

		Chemistry	Microbiology	Both
Qualitative	**Single lab**	5, 8	1.1, 1.3, 2.2, 3, 4, 6	
	Multi lab	5	1.1, 1.3, 1.4, 2.2, 3, 4, 6	
Quantitative	**Single lab**	2.3, 2.4, 5, 7, 8	1.1, 1.3, 1.5, 2.2, 3, 4, 6	
	Multi lab	2.1, 2.5, 5	1.1, 1.3, 1.4, 2.2, 3, 4, 6	1.2

In the following sections, procedures from ISO are numbered 1.1 to 1.5. Similarly, those from AOAC are numbered 2.1 to 2.5, and those from other organizations are numbered from 3 onwards.

Support with validation and other laboratory activities

As mentioned above, method validation is just one aspect that laboratories have to consider when assuring the quality of the results that they produce. There are a range of issues that need to be considered. Campden BRI has extensive experience, not only of managing its own laboratories, but of supporting others – as well as running the Campden Laboratory Accreditation Scheme (CLAS) and a range of proficiency schemes.

Please contact us if you would like to discuss any aspect of laboratory management and performance, and in particular with regards to method validation:

- Selection and implementation of appropriate procedures
- Statistical analysis of results
- Provision of reference materials or results from reference methods
- Course and publication on method validation

Please send any queries to info@campdenbri.co.uk and one of our laboratory support team will get in touch to help.

Descriptions of procedures

1. ISO procedures

1.1ISO 16140:2003- Microbiology of food and animal feeding stuffs - Method validation

- Scope:
  - Validation of alternative (proprietary) methods
  - Microbiological analysis of food, animal feeding stuffs and environmental and veterinary samples
  - Quantitative and qualitative methods, separate protocols

- Approach
  - Comparison of alternative and reference methods
  - Separate protocols for:
    - Single-lab method comparison study and
    - Interlaboratory study (at least 8 laboratories)

- Remarks
  - Under revision (**Target publication date:** 20-10-2014). Two parts: Vocabulary and Protocol
  - Favoured by MicroVal, AFNOR and NordVal

- Test characteristics assessed:

Qualitative methods:	Quantitative methods:
1. Selectivity (inclusivity/ exclusivity) 2. Relative accuracy 3. Detection level 4. Relative sensitivity 5. Relative specificity 6. The agreement between the methods	1. Selectivity (inclusivity/ exclusivity) 2. Lowest validated level with satisfactory precision 3. Linearity 4. Precision a. Repeatability b. Reproducibility 5. Relative accuracy 6. Bias 7. Uncertainty of the method 8. Detection and quantification limit

1.2 ISO 5725 Accuracy (trueness and precision) of measurement methods and results

- Scope:
  - Assess accuracy of measurement methods and results
  - Applied to a very wide range of materials, including liquids, powders etc.
  - Quantitative methods

- Approach
    - Assessment of “Trueness” needs “accepted reference values” from known samples or reference methods
    - Separate protocols for:
        - Estimating the precision of measurement methods by means of a collaborative interlaboratory study(at least 8 laboratories)
        - Estimating of intermediate precision within a specific laboratory or by an interlaboratory experiment
        - Estimating the bias of a measurement method and the laboratory bias

- Remarks
    - Consists of 6 parts
    - Includes practical guidance and step-by-step procedures for experimental design, implementation, and statistical analysis (ISO/TR 22971:2005)

- Test characteristics assessed:

Quantitative methods:
1. Precision a. Repeatability b. Reproducibility c. Intermediate precision 2. Bias (Trueness)

1.3 ISO/TR 13843:2001 Water quality - Guidance on validation of microbiological methods

- Scope:
    - Validation of microbiological methods for different types of water
    - Methods covered:
        - Presence/absence
        - MPN
        - Colony count
        - Direct (microscopic) count
    - Not applicable to the validation of so-called “rapid” or “modern” methods
    - Quantitative and qualitative methods

- Approach
    - Primary validation; covers development of
        - In-house method
        - A variant of existing standard method
    - Assessment of “Trueness” needs “accepted reference values” from known samples or reference methods.

- Remarks
    - Under revision ISO/AWI 13843: Water quality -- Requirements for establishing performance characteristics of quantitative microbiological methods was registered as a new ISO project 22 Jan 2013.

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Sensitivity 2. Specificity 3. False positive rate 4. False negative rate 5. Efficiency 6. Selectivity	1. Precision a. Repeatability b. Reproducibility 2. Recovery 3. Robustness 4. Linearity

1.4 ISO 17994:2004 Water quality - Criteria for establishing equivalence between microbiological methods

- Scope:
    - Evaluates the average relative performance of two methods, one of which may but need not be a standard or reference method
    - Quantitative and qualitative methods
    - Provides no solution to directly compare a quantitative with a detection method

- Approach
    - Interlaboratory study (at least 6 laboratories)

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Poisson index of dispersion (a measure of difference in detection rates)	1. Relative differences 2. Mean relative difference 3. Standard deviation of the relative difference 4. Expanded measurement uncertainty

1.5 ISO 8196-3:2009 (IDF 128-3: 2009) Milk - Definition and evaluation of the overall accuracy of alternative methods of milk analysis - Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis

- Scope:
    - Evaluation and validation of alternative quantitative method
    - Microbiological analysis of all milk components including somatic cells
    - Quantitative methods

- Approach
    - Single-lab method comparison of alternative and reference methods

- Test characteristics assessed:

Quantitative methods:
1. Precision a. Repeatability b. Reproducibility 2. Accuracy 3. Conformity tests 4. Linearity

2. Official Methods of Analysis of AOAC INTERNATIONAL, 19th Edition (2012)

2.1 Guideline for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis (Appendix D)

- Scope:
  - Developed for chemical studies, but some parts may be applicable to other types of collaborative studies
  - Quantitative method

- Approach
  - Assessment of "Trueness" needs "accepted reference values" from known samples or reference methods

- Test characteristics assessed:

Quantitative methods:

1. Mean
2. Percent recovery
3. Precision
   a. Repeatability
   b. Reproducibility
   c. HORRAT
4. Accuracy (if applicable)

2.2 AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (Appendix J)

- Scope:
  - Validation of alternative methods
  - Microbiological analysis of food, animal feeding stuffs and environmental and veterinary samples.
  - Quantitative and qualitative methods, separate protocols

- Approach
  - Comparison of alternative and reference methods
  - Separate protocols for:
    - Single-lab method comparison study and
    - Interlaboratory study (at least 10 laboratories)

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Selectivity (inclusivity/ exclusivity) 2. Sensitivity rate 3. Specificity rate 4. False negative rate 5. False positive rate	1. Selectivity (inclusivity/ exclusivity) 2. Precision a. Repeatability b. Reproducibility 3. Mean difference between Candidate and Reference methods (where applicable) 4. Bias 5. Robustness

2.3 AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals

- Scope
  - Single laboratory validation
  - Quantitative methods

- Approach
  - Comparison of alternative and reference methods (usually a reference material or standard)

- Remarks
  - This is one of three parts of "Appendix K: Guidelines for Dietary Supplements and Botanicals". The other two parts ("AOAC Guidelines for Validation of Botanical Identification Methods" and "Probability of Identification: A Statistical Model for the Validation of Qualitative Botanical Identification Methods") are of limited interest in food and drink laboratories and are not described here

- Test characteristics assessed:

Quantitative methods:
1. Accuracy 2. Precision a. Repeatability b. Reproducibility c. Intermediate precision d. HORRAT 3. Bias 4. Standard measurement uncertainty

2.4 AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation (Appendix L)

- Scope
    - Single laboratory validation utilizing the available SPIFAN matrices
    - Quantitative methods

- Approach
    - Precision data using SRM (standard reference material)1849a should be included for all methods
    - Assessment of "Trueness" may be done by comparison to SRM1849a
    - Recovery will be determined from an appropriate sampling of SPIFAN matrices, either
        - unfortified (preferably)
        - fully fortified products may be used
    - Comparison to reference methods is not required as matter of routine

- Test characteristics assessed:

Quantitative methods:

1. Linearity
2. Accuracy
3. Precision
    a. Repeatability
    b. Reproducibility
    c. Intermediate precision
4. Detection and quantification limit

2.5 Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices (Appendix M)

- Scope
    - Validation of ELISA based method for food allergens
    - Quantitative methods

- Approach
    - Interlaboratory study (at least 8 laboratories)

- Remark
    - A single-laboratory validation study of ELISA-based allergen detection method should be carried out in-house according to available guidelines
    - Assessment of "Trueness" may be done by comparison to available reference material
        - For egg detection NIST egg powder (NIST RM-8445)
        - For milk detection NIST nonfat milk powder (NIST RM-1549)

- Test characteristics assessed:

Quantitative methods:

1. Accuracy (if applicable)
2. Precision
    a. Repeatability
    b. Reproducibility
    c. HORRAT
3. Detection and quantification limits

3. FDA - Guideline for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods (September 2011)

- Scope:
    - Applies to detection methods of all microbial analytes associated with foods
    - Validation of alternative methods
    - Quantitative and qualitative methods

- Approach
    - Four levels of performance are defined with different numbers of labs
      Level 1: one lab
      Level 2: two labs
      Level 3: multiple labs
      Level 4: equivalent to AOAC full validation

- Remarks
    - Accepted in the US region

- Test characteristics assessed are:

Qualitative methods :	Quantitative methods:
1. Selectivity (inclusivity/ exclusivity) 2. Sensitivity rate 3. Specificity rate 4. False negative rate 5. False positive rate	1. Selectivity (inclusivity/ exclusivity) 2. Precision a. Repeatability b. Reproducibility 3. Mean difference between Candidate and Reference methods (where applicable) 4. Bias 5. Robustness

4. NordVal - Protocol for the Validation of Alternative Microbiological Methods (March 2009)

- Scope:
  - Validation of alternative (proprietary) methods
  - Microbiological analysis of food, water, animal faeces, feed, samples from equipment and processing area and environmental samples
  - Quantitative and qualitative methods, separate protocols

- Approach
  - Comparison of alternative and reference methods
  - Separate protocols for:
    - Single-lab "method comparison study" and
    - Interlaboratory study (at least 8 laboratories)

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Selectivity (inclusivity/exclusivity) 2. Relative accuracy 3. Detection level 4. Relative sensitivity 5. Relative specificity 6. The agreement between the methods	1. Selectivity (inclusivity/exclusivity) 2. Lowest validated level with satisfactory precision 3. Precision a. Repeatability b. Reproducibility 4. Standard measurement uncertainty

5. NordVal - Guide in Validation of Alternative Proprietary Chemical Methods (May 2010)

- Scope:
  - Validation of alternative (proprietary) methods
  - Applied to chemical methods (test kits)
  - Quantitative and qualitative methods, separate protocols

- Approach
  - Comparison of alternative and reference methods
  - When no reference method available use certified reference materials, control materials and/or spiked samples at different levels in various matrices
  - Separate protocols for:
    - Single-lab method comparison study and
    - Intermediate study (at least one additional laboratory)

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Limit of detection 2. Accuracy 3. Inclusivity 4. Sensitivity 5. Specificity 6. False positive rate 7. False negative rate 8. The agreement between the methods	1. Limit of quantification 2. Specificity 3. Inclusivity 4. Repeatability 5. Trueness 6. Recovery

6. AFNOR – Validation of Analysis Methods; Application to Water Microbiology (Oct 2013)

- Scope:
  - Validation of alternative methods
  - Applied to microbiological analysis of water (all types of water)
  - Quantitative and qualitative methods, separate protocols

- Approach
  - Comparison of alternative and reference methods (when it exists; natural or artificial samples or on reference materials)
  - Separate protocols for:
    - Single-lab method comparison study and
    - Intermediate study (at least 8 laboratories)

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Selectivity (inclusivity/ exclusivity) 2. Relative accuracy 3. Detection level 4. Relative sensitivity 5. Relative specificity	1. Selectivity (inclusivity/ exclusivity) 2. Linearity 3. Precision a. Repeatability b. Reproducibility 4. Relative accuracy 5. Detection and quantification limits 6. Bias

7. APVMA - Guidelines for the Validation of Analytical Methods for Active Constituent, Agricultural and Veterinary Chemical Products (October 2004)

- Scope:
  - Validation of alternative methods
  - Applied to agriculture and chemical products
  - Not suitable for analytical methods for residue analysis, biological and biotechnological products
  - Quantitative method

- Approach
  - Comparison of alternative and reference methods
  - Assessment of "Trueness" needs "accepted reference values" from known samples or reference methods

- Remarks
  - Accepted in Australia

- Test characteristics assessed:

Quantitative methods:

1. Precision
   a. Repeatability
   b. Reproducibility
2. Accuracy
3. Linearity
4. Detection and quantification limit

8. NMKL - Manual for NMKL Peer-Verification (Interlaboratory Verification) of Methods (2010)

- Scope:
  - Describing, validating and testing the alternative method
  - Intended only for chemical methods
  - Quantitative and qualitative methods

- Approach
  - Comparison of alternative and reference methods (reference materials, other known samples, standard addition)

- Remarks
  - Accepted in Norway

- Test characteristics assessed:

Qualitative methods :	Quantitative methods:
1. Specificity 2. False positive rate 3. False negative rate	1. Precision a. Repeatability b. Reproducibility 2. Relative accuracy 3. Trueness 4. Recovery 5. Detection and quantification limit

Glossary

List of acronyms

AFNOR - The French National Association for Standardisation

AOAC - Association of Official Analytical Chemists

APVMA - Australian Pesticides and Veterinary Medicines Authority

FDA - Food and Drug Administration

ISO - International Organization for Standardization

MicroVal - A European certification organisation for the validation and approval of alternative methods for the microbiological analysis of food and beverages

NIST - National Institute of Standards and Technology

NMKL - Nordic Committee on Food Analysis (Nacionalinė moterų krepšinio lyga)

NordVal - A Nordic system for validation of alternative microbiological methods

SPIFAN - Stakeholder Panel on Infant Formula and Adult Nutritionals

Statistical terms

Accuracy	The closeness of agreement between a test result and the accepted reference value
Alternative method	A method of analysis that demonstrates or estimates, for a given category of products, the same analyte as is measured using the corresponding reference method
Analyte	Component measured by the method of analysis. In the case of microbiological methods, it is the microorganism or associated by-products
Bias	The difference between the expectation of the test results and an accepted reference value

Certified Reference Material	Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures
Conformity test	The process used to show that a product, service or system meets specified requirements
Expanded measurement uncertainty	Product of a combined standard measurement uncertainty and a factor larger than the number one
False negatives and false positives rate	The false negative rate is the probability that the test is negative for samples that contain the analyte The false positive rate is the probability that the test is positive for samples that do not contain the analyte at the screening level
HORRAT value	The ratio of the reproducibility relative standard deviation, expressed as a percent, to the predicted reproducibility relative standard deviation expressed as a percent predicted reproducibility relative standard deviation = $2C^{-0.1505}$ where C is the estimated mean concentration
Interlaboratory study	A study of the method's performance using common samples in several labs and under the control of the organising lab
Intermediate precision	The precision determined from replicate determinations conducted within a single laboratory not simultaneously, e.g. on different days, with different instruments, by different analysts etc.
Intermediate study	A study of the proprietary method's performance by at least one additional independent laboratory
Limit of detection	Measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence
Limit of quantification	The lowest average concentration of an analyte that may be measured and quantified with known uncertainty, under the experimental conditions of the method considered
Linearity	The ability of the method to obtain test results proportional to the concentration of analyte
Method comparison study	A study performed by the organizing laboratory of the alternative method against the reference method

Negative deviation	The proprietary method presents a negative deviation if it gives a negative result when the reference method gives a positive result
Positive deviation	The proprietary method presents a positive deviation if it gives a positive result when the reference method gives a negative result
Precision	The degree of agreement between independent analysis results obtained under specific circumstances
Qualitative method	A method of analysis whose response is either the presence or absence of the analyte in a certain amount of sample
Quantitative method	A method of analysis whose response is the amount of the analyte measured either directly or indirectly in a certain amount of sample
Recovery	The proportion of the known amount of an analytical parameter which is measured
Reference method	A method which is internationally recognised and accepted (e.g. NMKL, ISO, CEN and AOAC International methods, methods given in EU/national legislation and certain national standards of equivalent standing)
Relative sensitivity	The ability of the alternative method to detect the analyte compared to the reference method
Relative specificity	The ability of the alternative method not to detect the target microorganism when it is not detected by the reference method
Repeatability	The closeness of agreement between successive and independent results obtained by the same method on identical test material under the same conditions (apparatus, operator, laboratory and short intervals of time)
Repeatability limit (r)	The value less than or equal to which the absolute difference between two tests results obtained under repeatability conditions is expected to be with a probability of 95%
Reproducibility	The closeness of agreement between single test results on identical test material using the same method and obtained by operators in different laboratories using different equipment
Reproducibility limit (R)	The value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions is expected to be with a probability of 95%

Robustness	A measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage
Selectivity	of a) the inclusivity: detection of the target microorganism from a wide range of strains, and b) the exclusivity: the lack of interference from a relevant range of non-target microorganisms
Standard measurement uncertainty	A parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurand
Trueness	The closeness of agreement between the average value obtained from a large set of test results and an accepted reference value

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