

VALIDATION OF MICROBIOLOGICAL TESTING METHODS

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Abstract: Method-validation is a method which proofs that a given analytical process, when applied well, produces findings which are suitable for a purpose and of acceptable standard. A process whereby an authentic information is given on an analytical method duly applied and enough to meet the needs and acceptable standards, is known as method-validation. The goal to validate a method therefore, is to make the findings of the method-validation reliable throughout the course of the study. With the above in mind, validating a method therefore, is for the purpose of making the obtained results reliable in the study. A laboratory should authenticate non-standard methods and laboratory-designed/developed methods. Validation studies should also be done when new equipment steps into the work or an important change occurs along the new chemicals. The method is carried out by a new personnel. A validation method that has not been used for a long time, demanded to be used during a study, is thought to affect the laboratory results. Laboratories need to make a policy and procedures for the selection and the use of analytical methods. The method will successfully meet or exceed the minimum standards recommended for accuracy, precision, selectivity, sensitivity, reproducibility, and stability.

Key words: Accuracy, precision, reproducibility, sensitivity, selectivity, validation.

Mikrobiyolojik Test Metotlarının Doğrulanması (Validasyonu)

Özet: Metot doğrulanması, amaca en uygun ve kabul edilebilir nitelikte sonuçları üretecek analitik metodun ortaya çıkarılması sürecidir. Doğrulanmış metodun hedefi bu metotla yapılmış sonuçlara güvenilirlik sağlamaktır. Laboratuvar, standart olmayan metotları, laboratuvar tarafından geliştirilen veya tasarlanan metotları geçerli kılmalıdır. Doğrulama çalışmaları yeni bir cihaz devreye girdiğinde ya da yeni kimyasallarda önemli bir değişiklik olduğunda da yapılmalıdır. Yöntem yeni bir personel tarafından uygulanır. Uzun süredir kullanılmayan ancak kullanılmasına gereksinim duyulan bir doğrulama yönteminin laboratuvar sonuçlarını etkilediği düşünülür. Laboratuvarların, analitik metotların seçimi ve kullanımı için bir yöntem ve prosedür belirlemeleri gereklidir. Metot, kesinlik, doğruluk, seçicilik, hassasiyet, tekrar edilebilirlik ve kararlılık için gerekli minimum standartları başarılı bir biçimde karşılamalı ya da aşmalıdır.

Anahtar kelimeler: Doğruluk, kesinlik, tekrar edilebilirlik, hassaslık, seçicilik, validasyon.

Introduction

Analytical methods are one of the basic tools in laboratory and have been defined or classified in various ways. The basic requirement should be that this method must meet the desired goal, in other words, be practical and suitable for the intended use. Methods can also be loosely classified as official, reference, screening or rapid, in-house and automated methods according to their purposes or their administrative propriety (Garfield *et al.* 2000). A method that is acceptable for its intended purpose is normally authenticated by a process known as Method Validation. Validating a method, therefore, serves as a way of authenticating that the analytical method receives acceptance for the targeted goal (McCully & Lee 1980, Green 1996). Validation or substantiation is the practical demonstration and the tendering of objective facts that the particular requirements for an intended use are met. Validation means, testing and confirmation towards providing a

standard proof that these specific requirements are for a particular use. The laboratory also confirms that standard methods used outside their targeted scope and amplifies/modifies the methods to show that they are right for the intended use (Elder *et al.* 1997, White *et al.* 2001, NELAC 2007, EA 2012).

In general, specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness should constitute the methods submitted to the authorities. Submissions should embody works on specificity, linearity, range, detection limit, precision, robustness and quantitation. The procedures for authentication of a method mustn't be alienated from the actual development of the method conditions, since the initiators will not know, whether the method conditions are acceptable until validation studies are proved.

Table 1. Method validation tools (Garfield *et al.* 2000).

Validation Parameter	Description and validation characteristics
Spike or recovery	Adding clear quantities of pure substance to portions of previously analyzed material, and repeating the analysis using the same reagents and technique should enable recovery data.
Method comparison	Two different methods should be employed in analyzing standard or known materials.
Linearity	Spanning the range of the methods, with the analyte concentrations, are the materials with which they are determined. With using the least squares method, a regression line could be computed when linearity is not attainable, an algorithm specific for that analyte/matrix combination may be used.
Reference standards and standard reference materials	A standard of stated purity from which measurements are carried out at that location are derived. A substance with one or more properties correctly established for use in calibration in order to assess a method or for giving values to materials is known as standard reference material.
Certified reference materials	This is a material of the highest metrological quality in existence, one or more in number and whose values are authenticated by a technically standard procedure, followed by or linked with a certificate issued by the certified authority.
Duplicates and replicates	Repeated, independent determinations of the similar test sample through the same analyst at essentially the correct time and conditions of the entire analysis. Replicates may be considered as repetitions of the determinative step only.
Blanks	The reagent blank is the simplest type of blank, that the method is fully performed except for addition of the test portion. This tests the purity of reagents and also detects contamination of the analytical system from any source. Simulated test material used at times can give a better performance of blank determinations.
Collaborative study	This is the analysis by some laboratories with identical sample sets and can cover the range of applicability of a method found before to be good and practical, to record the characteristics of a method regarding accuracy, precision, sensitivity, range, specificity, limit of detection together with the limit of reliable measurement, selectivity, and practicality.
Validation by other laboratories	Validation of the method may be done by one or more outside laboratories.

The mechanisms of developing a method are also parts and parcels of validating it since the developer will not be able to know if the conditions as specified by the method would be adopted until validated. Findings of the validation studies may show that a change in the procedure is inevitable, and may need then revalidation processes may bringing out the necessity to make changes, requiring revalidation. For each validation study, the main method parameters are established and used for all the other subsequent validation steps for each and every validation, the main parameters established and used for all the future validation steps (Green 1996). The laboratory records all the results obtained, the procedure used for validation and issues a statement certifying that the method fits for the intended use. The statement as to whether the validation fit the purpose should be issued by the laboratory based on the results recorded (NELAC 2007). Table 1 lists the validation parameters and their detailed descriptions and validation properties.

Microbiological Method Validation

Technology for microbiological analyses is widespread (NELAC 2007). Isolation, enumeration, detection or identification of microorganisms and/or their metabolites or spotting of the presence or absence of growth in materials and media are all known and included in microbial testing methods (CDER 2015). These methods are grouped into either qualitative methods that demonstrate the presence or absence of the target microorganism, directly or indirectly in a defined quantity of test material, or quantitative methods that identifies the number of microorganisms available through direct enumeration (colony forming units) or indirectly (most probable number counts, color absorbance, impedance) in a standard quantity of material (Wills 2000, NordVal 2009, Eurachem 2013). Some qualitative microbiological test methods, such as where the result is expressed in terms of detected/not detected and confirmation and identification procedures, should be confirmed by

determining, where suitable, the specificity, relative exactitude, positive and negative deviations, limit of detection, matrix effect, repeatability and reproductivity. In the case of quantitative microbiological test methods, specificity, sensitivity, relative acuteness, positive and negative deviations, repeatability, reproducibility and the challenges of determination within the framework of a defined variability should be considered and, if required, quantitatively determined (ISO/IEC 17025:2005, ISO 7218:2007).

With the use of a reference method, demonstration should be made in laboratory for its competence to determine if it meets the performance characteristics prescribed both, in the national and international standards. Microbiological researches could be done using optional (rapid) methods like immunological, molecular biological or instrumental. The authentication of these methods takes into account the assessment of their equality to the corresponding reference method. When it comes to microbiology, using correct quality controls is very important, since the translation of the performance characteristic to microbiological examination is not normally guaranteed and depends on the test matrix. Validating microbiological methods should not be based on the same principle as in chemical methods (Golcteger 2001, ISO 16140:2003). The actual test conditions should be reflected on by the test methods when validating the microbiological test. Achievement could be attended applying purely natural contaminated products or products mixed with a predetermined level of contaminating organisms (Wills 2000, Sartory 2005, AOAC 2006). Matrix differences should be considered when testing various types of samples. Appropriate statistical methods should be used when validating the results (Wills 2000). In the case of the modification of a version, a method is needed just as in the original method and requires comparisons, using the replicates to ensure that this is the case (Eurachem 2013). Statistical validity must be adhered to experimental design and analysis of the results (Wills 2000). Even on the completion of validation, an operator will still have to verify regularly that the documented performance can be reached, e.g. using spiked samples or reference materials involving the relevant matrices (NordVal 2009, ISO/IEC 17025:2005). Laboratories should be able to show that the method is performing in their laboratory environment (Nolard & Chasseur 2004). They need to keep documented evidence that the method is performing as expected, and that a decision has been made by the laboratory to accept the performance. Method information given by method validation on specification of performance is not only on the recovery and enumeration of the target organism(s), but is also for the analytical requirements of the method in practice (e.g. incubation temperature and time, media preparation and storage conditions and sample storage or afore-treatment) (Kromidas 2000). Recovery efficiency is attached to the main information, upper and lower working (detection) bounds, selectivity and specificity (inaccurate-positive and negatives), counting uncertainty

(methodological and analyst) and a general appropriation of precision. As the data is to give the first assessment of performance of a new or altered method, it is highly emphasized that analysts with good experience in microbiological methods carry out the work (COFRAC 2004). The basic parameters for a bio-analytical method authentication are accuracy, precision, selectivity, reproducibility, stability, sensitivity, repeatability, limit of detection and of quantification.

Accuracy: The exactitude of an analytical method explains how close the mean test results obtained are to the real value of the analyte (CDER 2015). The replicate analysis of samples containing known amounts of the analyte determines the accuracy (Riley 2003, USPC 2003). As the results expected are normally centered on the existing method, it is essential to test the accuracy by making a comparison between the old and new methods (PDA 2000, MAF 2002). Spiking trials are normally applied to get accuracy data. The average and standard deviation of a number of repeated tests with spiked materials must be obtained and comparison made with the characterized value for the material used as the reference. When making spike trials, spike addition should be made early in the analysis to enable that extraction efficiency is included in the results (FAO 1999, Thompson *et al.* 2002). When reference material is not available for spiking trials, the parameter is difficult to assess. Accuracy can also be established for stuffs with different matrices (Kromidas 2000). Even if it is one, a pure reference culture under the expected environmental terms should be applied and the test method findings should be compared with a reference or standard method (ISO/IEC 17025:2005, Kromidas 2000).

Precision: Analytical precision method explains the closeness of individual measures of an analyte when the procedure is done again and again on various aliquots with a homogeneous volume of biological matrix (CDER 2015). Precision is very much important as an identification method because trending isolates can be hard if the same organism is given different identities at each time it is isolated (PDA 2000, Eurachem 2014).

Repeatability: Repeatability must be calculated when authenticating a method being a measure of agreement of replicate tests carried out on the same material in the same laboratory by the analysts (MAF 2002, Eurachem 2014).

Reproducibility: Reproducibility must also be calculated during validation and is a test to prove its agreement with the tests done by other laboratories. Generally, the expectation is that within a laboratory, variabilities would be less among laboratories variations. Under one laboratory confirmation studies, reproducibility could be a measure to test the agreement among tests done in different days by different experts (MAF 2002, Eurachem 2014).

Selectivity/Specificity: This is the capability of an analytical proof to verify and quantify the analytes among other components in the sample. In the case of selectivity,

analyses of virgin samples of the exact biological material must be obtained from not less than six sources. Each and every one of the blank sample must be tested for interference and selectivity made to ensure the lower limit of quantification (MAF 2002, Eurachem 2014).

Sensitivity: Sensitivity of the test method should be made and can be explained as the limit of accurate measurement. This is to show the limit that a method can be discriminated, with a large measure of trust, between and above levels below several critical values close to zero. Sensitivity is the effectiveness of the gradient response curve or the change in instrument response to correspond with the change in analyte concentration (FAO 2001, MAF 2002, Eurachem 2014).

Stability: Procedures have to measure the stability of the analytes when collecting and handling samples, after long and (frozen at the intended storage temperature) short-term (bench top, room temperature) storage, and going through freezing and thawing cycles along with the analytical processes. The terms applied in stability trials must reflect conditions supposedly to be met during the real material-handling and analysis. The processes must also take into account the assessment of analyte stability in stock solution (CDER 2015).

References

1. AOAC, 2006. *Presidential Task Force on Best Practice for Microbiological Methodology*, US FDA, Appendix G-STWG Executive Summary 7-16-06 7-16-06: 1-5.
2. CDER (U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research Center for Veterinary Medicine (CVM)), 2015. Guidance for Industry Bioanalytical Method Validation. <http://www.fda.gov/downloads/drugs/guidancecompliance/regulatoryinformation/guidances/ucm386366.pdf>, (Date accessed: 15 June 2016).
3. COFRAC (French Committee for Accreditation), 2004. Guidelines for Method Validation in Medical Biology, Document LAB GTA (04), <https://www.cofrac.fr/en/activites/laboratoires.php>. (Date accessed: 12 June 2016).
4. EA (European co-operation for Accreditation), 2012. The Scope of Accreditation and Consideration of Methods and Criteria for the Assessment of the Scope in Testing, EA-2/05, Publication Reference, 13 pp.
5. Elder, B.L., Hansen, S.A., Kellogg, J.A., 1997. CUMITECH 31: verification and validation of procedures in the clinical microbiology laboratory. Washington DC: American Society for Microbiology.
6. Eurachem, 2013. Accreditation for Microbiological Laboratories, Eurachem Guide, 32 pp.
7. Eurachem, 2014. The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, Eurachem Guide, 70 pp.
8. FAO, Joint FAO/IAEA Expert Consultation, 1999. Guidelines for Single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals.
9. FAO, Joint FAO/WHO, 2001. Proposed guidelines and working instructions to aid the implementation of the criteria approach to the selection of methods of analysis for codex purposes, Food Standard Programme, Report on the twenty-third session of the Codex Committee on the methods of analysis and sampling, Alinorm 01/23.
10. Garfield, F., Klesta, E. & Hirsch, J. 2000. *Quality Assurance Principles for Analytical Laboratories*. AOAC International, Third Edition, USA, 113-129.
11. Golcteger, S. 2001. Microbiological examination and proficiency testing in dairy laboratories. *Archives of Industrial Hygiene and Toxicology*, (52): 61-67.
12. Green, M. 1996. A Practical Guide to Analytical Method Validation. *Analytical Chemistry*, (68): 305A-309A.
13. ISO 16140:2003. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods, http://www.iso.org/iso/catalogue_detail.htm?csnumber=30158. (Date accessed: 12 June 2016).
14. ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories, http://www.iso.org/iso/catalogue_detail.htm?csnumber=39883. (Date accessed: 15 June 2016).
15. ISO 7218:2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations,

Limit of Detection/Limit of Quantification: This is the minimum amount of organisms which can be identified and counted. The limit of detection is especially necessary in the case of qualitative tests as in sterility which must be able to identify a single viable organism. Nonetheless, practically testing this ability is not easy because of problems associated with preparing a sample which have only one viable organism. When a sample with less numbers of organisms are used (e.g., 1-10 CFU), it should be enough to assess the limit of detection (PDA 2000, Riley 2003, USPC 2003).

Conclusions

A validation method could be defined as an arrangement of procedures and the documentation setting out test performance characteristics. It is therefore, these performance characteristics or the validation parameters of the method which establish its appropriateness for the purpose it was meant for. The characteristics and parameters, spell out what this method could achieve in an optimized laboratory conditions of solution texture, instrumental settings, analyte isolation, including the other features in which such an experiment is done. Bringing in certain validation parameters in any validation agreement is guided by its usage, samples test and the aim of this method, taking cognizance of both the local and the international rules and regulations in play.

- <http://www.iso.org/iso/home>. (Date accessed: 15 June 2016).
16. Kromidas, S., 2000. Handbook of validation in analysis, Verlag Wiley-VCH, Weinheim, ISBN 3-527-29811-8.
 17. MAF (Food Assurance Authority), 2002. A guide for the validation and approval of new marine biotoxin test methods, Shellfish Quality Assurance Programme, A Guide to the Validation of New Test Methods, Seafood Industry Agreed Guidelines Issue 1: P2. Wellington, 1-22.
 18. McCully, K.A. & Lee, J.G. 1980. Optimizing Chemical Laboratory Performance Through the Application of Quality Assurance Principle, Association of Official Analytical Chemists, Arlington, VA, 73 pp.
 19. NELAC, 2007. Microbiological Testing, Draft Interim Standard, Vol 1, Module 5, www.nelac-institute.org, (Date accessed: 12 June 2016).
 20. Nolard, N. & Chasseur, C. 2004. Validation of microbiological and chemical inspections for the workplaces, Programmes PS 50/47 and PS/50/48: final report, 1-93.
 21. NordVal, 2009. Food microbiology protocol for the validation of alternative methods, <http://www.nmkl.org/dokumenter/nordval/NordValProtocol.pdf>. (Date accessed: 15 June 2016).
 22. PDA, 2000. Evaluation, Validation and Implementation of New Microbiological Testing Methods, Technical Report No. 33, PDA Journal of Pharmaceutical Science and Technology, Supplement TR33, 54 (3).
 23. Riley, B.S. 2003. Rapid microbiology methods in the pharmaceutical industry, American Pharmaceutical Review, The Review of American Pharmaceutical Business and Technology, <http://www.americanpharmaceuticalreview.com/Featured-Articles/113094-Rapid-Microbiology-Methods-in-the-Pharmaceutical-Industry/>, (Date accessed: 22 November 2016).
 24. Sartory, D.P. 2005. Validation, verification and comparison: Adopting new methods in water microbiology. *Water SA*, 31 (3): 393-396.
 25. Thompson, M., Ellison, S.L.R. & Wood, R. 2002. Harmonised guidelines for single-laboratory validation of methods of analysis. *Pure and Applied Chemistry*, (74): 835-855.
 26. USPC Inc., 2003. *Microbial limits tests*. USP 26, Rockville, MD, 2006 pp.
 27. White, V.R., Alderman, D.F. & Fasion, C.D. 2001. *Procedures and General Requirements, National Voluntary Laboratory Accreditation Program Office of Standards Services Technology Services*, NIST Handbook 150, 60p.
 28. Wills, K., 2000. *Evaluation, Validation of Implementation of New Microbiological Testing Methods, A Brief Review of the Highlight of PDA Technical Report No.33*, Celsis Ltd, UK.

