Recommendation for validation and verification of laboratory examination procedures in medical laboratories

Date of issue: 1 March 2021

Authors: Z. Plzák, J. Kratochvíla, B. Friedecký, L. Šprongl

Table of contents

1. Introduction 3
2. Abbreviations 3
3. Subject of validation in the laboratory 3
4. Subject of verification in the laboratory 4
5. Terminology 4
6. Why validation/verification is necessary 4
7. Who performs validation/verification 5
7.1. Manufacturers of diagnostics 5
7.2 Professional organization of analysts 5
7.3 Medical laboratories 5
8. When validation is performed 5
9. When verification is performed 5
10. Scope of validation and verification 6
10.1 Verification of validated qualitative tests 6
10.2 Verification of validated quantitative tests 6
10.3 Validation of modifications made to the originally validated method 7
10.4 Quantitative examinations developed by the laboratory (in-house) 7
11. Establishing a validation/verification plan 7
12. Implementation of the validation/verification plan 7
13. Performance characteristics of the laboratory examination procedure 8
13.1 Aspects closely related to validation/verification 9
14. Documentation 9
15. Release and implementation 9
16. Summary of validation principles 10
17. Summary of verification principles 10
18. References 11

1. Introduction

It has been 10 years since the last edition of the Recommendation for Validation and Verification of Analytical Methods in Clinical Laboratories was issued. During this time, new versions of the documents on which this Recommendation is based have been published. First of all, a new standard EN ISO 15189 [1] has been implemented, according to which medical laboratories are accredited and which specifies the requirements for the validation and verification of the analytical methods used, and laboratory examination procedures. There is also a new, revised edition of the EURACHEM Guide [2, 3] dealing with this topic and other documents discussing in detail the validation and verification of methods in laboratories [4, 5, 6, 7]. Last but not least, the current position paper of the Accreditation and ISO/CEN Standards Working Group of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) [8] on validation and verification of laboratory examination procedures in medical laboratories has been published, on which this new version of the Recommendation is based.

2. Abbreviations

AOAC	Association of Official Analytical Chemists
CEN	Comité Européen de Normalisation (European Committee for Standardization)
CLIA	Clinical Laboratory Improvement Amendments USA
CRM	Certified Reference Material
EFLM	European Federation of Clinical Chemistry and Laboratory Medicine
EQA	External Quality Assessment
FDA	Food and Drug Administration USA
IVD MD	In vitro diagnostic – medical device
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JCTLM	Join Committee on Traceability in Laboratory Medicine
LGC	Laboratory of the Government Chemist
POCT	Point of Care Testing (Laboratory method performed at the bedside of a patient or the first contact with the patient)
SOP	Standard Operating Procedure
SRM	Standard Reference Material
WG-PRE	Working Group for the Preanalytical Phase

3. Subject of validation in the laboratory

Validation is defined in EN ISO 15189 [1] and in the Vocabulary of quality management systems [9] as the confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.

Validation confirms that the IVD MD measurement procedure/measurement system/product is capable of fulfilling the requirements imposed on it. In other words, that a laboratory examination performed by a given measurement method is capable of providing results - reproducible measurements within the required range, with the required limits of determination, precision, bias and uncertainty - to make possible appropriate diagnostic and therapeutic decisions.

In the medical laboratory, the vast majority of laboratory examination procedures used are already validated, produced by manufacturers of in vitro diagnostics in accordance with IVD Directive 98/79 EC (2017) or in accordance with FDA requirements. The medical laboratory should not use other than validated methods. The validation process is only performed by the laboratory if the laboratory examination procedures are [1, 8]:

a) Non-standard.
b) Designed or developed by the laboratory (in-house).
c) Validated laboratory examination procedures used outside their intended scope of use.
d) Validated laboratory examination procedures subsequently modified.

4. Subject of verification in the laboratory

Verification is defined in EN ISO 15189 [1] and the Vocabulary of quality management systems [9] as confirmation, through provision of objective evidence, that specified requirements have been fulfilled. That is to say, confirmation that the functional characteristics or legal requirements of a measurement system are achieved and confirmation by objective experimental evidence that the analytical performance data declared by the manufacturer, another laboratory or a reference institution are achieved in a given laboratory using a specific measurement system.

It is established that validated laboratory examination procedures used without modification shall be subject to independent verification in the laboratory before being introduced into routine use. [1]. The aim of verification is objective experimental evidence that the laboratory is capable of achieving the performance parameters (most often the basic analytical characteristics of the methods) that the manufacturer/manufacturer states (or is required to state) in its documentation. In laboratories, the concept of verification is the process of checking that the laboratory is able to achieve the declared method performance when implementing already validated methods, not a verification of the general fitness for purpose of the method. Although verification works with tools similar to validation and thus demonstrates the competence of laboratory personnel and the suitability of the equipment and environment in relation to the performance of a given measurement procedure, it is narrower in scope and far less demanding on the laboratory than validation.

Verification then means that the IVD MD measurement procedure/system/product is operational in a particular laboratory. In this guideline, we will only use the term verification in the context of products placed on the market in accordance with the principles of IVD Regulation 98/79 EC (2017). In other cases, the term validation is appropriate.

5. Terminology

The terminology used in this document is based on EN ISO 15189 [1] and the electronically published text "Metrology Terminology in the Clinical and Analytical Laboratory" (3rd revised and updated edition SEKK 2021), which is freely available at http://www.sekk.cz/terminologie/index.php. This document adopts selected concepts and terms as defined in the third edition of the International vocabulary of metrology VIM3 [10].

6. Why validation/verification is necessary

The results of analytical measurements have an extremely strong impact in practice. In the medical laboratory, they can have a decisive and sometimes fatal impact on the health, quality of life and sometimes even the life of the patient.

It is the professional duty of the analyst to perform measurements of sufficient quality. Validation/verification is the activity by which the laboratory ensures the quality of its data in routine operation. An activity, if properly performed, pays off for the laboratory in the routine use of the validated procedures.

Validation/verification provides the necessary data to estimate the uncertainty of the measurement (or confidence interval of the measurement results).

Accreditation, or formal confirmation of a laboratory's competence, requires the use of properly validated and verified methods (measurement systems).

7. Who performs validation/verification

7.1. Manufacturers of diagnostics

According to the IVD Regulation 98/79 EC, only validated measuring systems may be placed on the European Union market by the manufacturer. The procedure and scope of validation of IVD MDs is defined by EN 13612 [11].

For manufacturers marketing their products in the USA applies, that their products are validated within the scope of the FDA-led Bioanalytical Method Validation Guidance for Industry [12].

7.2 Professional organization of analysts

AOAC - aims to contribute to the global credibility of analytical results (and thus analytical chemistry and analytical chemists). This association has developed a number of validation, verifications, and certification programs and works with FDA-type institutions so that it also reaches directly into laboratory medicine. It refers to the analytical methods it publishes in its compendium as validated, because they have undergone a validation process involving interlaboratory comparisons. The AOAC has published its own guidance on how to verify analytical methods [4].

7.3 Medical laboratories

However, the ultimate responsibility for adequate validation or verification of the measurement procedure rests with the medical laboratory itself. The level of effort that the laboratory itself must make in validation or verification varies considerably depending on whether the subject of validation/verification is a new procedure developed in the laboratory or a standard or official method that has also undergone full interlaboratory validation and is commonly used in the field.

If the validation data comes from manufacturers' documentation and is supported by experiments in manufacturers' laboratories, professional organisations and calibration/reference laboratories, then the laboratory must continuously update and verify the data. The main tools to do this are:

- Compliance with standard operating procedures for products meeting the requirements of IVD 98/79 EC 2017.
- Performing regular quality control (internal and external quality control).
- Developing and revising SOPs, with a critical focus on evaluating the results of inter-laboratory comparisons and studies.
- Ongoing and continuous staff training.
- Continuous monitoring of documents and information provided by reference institutions and organisations such as JCTLM, LGC, AOAC.

The trend of modern analytical measurement is clear. To use validated test kits and validated analytical measurement systems and to shift the main focus of laboratories to estimation of measurement uncertainties, quality control, education, continuous monitoring of information and implementation of new knowledge into laboratory practice.

8. When validation is performed

- Prior to being introduced into routine use of a new analytical method/measurement system in the laboratory, where the method has not been validated by the manufacturer.
- When extending the use of an existing method to an additional purpose (e.g. extending the measurement to another type of biological material), beyond the scope of use declared by the manufacturer.
- When adopting a method (in house type) from another laboratory or publication.
- Before introducing a new (different) test kit.
- When there is a significant change in instrumentation.
- When quality control and EQA indicate a persistent serious problem.
- According to the validation plan.

9. When verification is performed

- Prior to being introduced into routine use of a new but already by the manufacturer validated standard method/measurement system.

- If quality control indicates a problem.
- According to the verification schedule, depending on the stability and robustness of the method.

10. Scope of validation and verification

The scope and depth of validation must always correspond to the need to obtain sufficient data to decide whether the method is indeed fit for its intended purpose [1]. In the case of verification, to confirm that the method is capable of performing under laboratory conditions (to exhibit analytical characteristics) such that it can be routinely used for its intended purpose. Validation/verification plans are determined by the nature of the method being validated/verified. Validation/verification of a qualitative method requires significantly less effort than validation/verification of a quantitative method. Manufacturer-validated methods - IVD MD products with CE marking and procedures verified by an inter-laboratory validation study require a lower level of verification than methods developed by the laboratory itself (i.e. in-house type) or methods substantially modified by the laboratory or adopted from another laboratory.

The scope of validation/verification shall be defined in the validation/verification plan.

In terms of validation/verification and its required scope, laboratory examinations can be divided into five groups:

1. Qualitative tests carried out using IVD products bearing the CE conformity mark.
2. Quantitative tests carried out using IVD products bearing the CE conformity mark.
3. Quantitative tests carried out using IVD products bearing the CE marking but using a modified procedure.
4. Quantitative tests developed by the laboratory (in-house).
5. Qualitative tests developed by the laboratory (in-house).

Validation and verification for the above groups of methods are different and are described in more detail in the following paragraphs. The validation must be as extensive as necessary to decide on the applicability of the method.

10.1 Verification of validated qualitative tests

For this group of methods, the only requirement is strict adherence to the manufacturers' operating procedures (final CLIA Rules Part V, available at http://www. westgard.com). This type of method should also have an established internal and external quality control program. According to the wording of EC Regulation 98/79, control materials are to be recommended, supplied and characterised by the IVD manufacturer according to ISO 15198 [13].

10.2 Verification of validated quantitative tests

These are methods that use devices that meet the requirements of IVD 98/79 EC and bear the CE mark. In this case the laboratory will limit itself to the verification of basic pre-validated parameters.

Most laboratory examinations fall into this category. CE marking of IVD products means confirmation of the conformity of their characteristics with the required ones, i.e. that they have been found to comply with the requirements of European Regulation 98/79 EC before being placed on the market. Thus, the IVD manufacturer has established a quality management system according to ISO 13485 [14] and its products have been validated by the procedures required by EN 13612 [11]. The fact that the laboratory uses IVD 98/79 EC-compliant products implies the following obligations for the laboratory, enabling it to claim manufacturer responsibility for the quality of the IVD MD product:

- The product must be used in accordance with its intended use as declared by the manufacturer.
- The user must follow the procedure indicated by the manufacturer (he must not modify this procedure in any way or in any way).
- The user must use the analytical measuring system specified by the manufacturer (he must not modify the analytical measuring system in any way or in any way).
- Instead of extensive validation, the user shall perform basic verification of the properties of these products using a simple verification plan.

The purpose of the verification of IVD methods is to confirm that the analytical and performance values obtained are in agreement with those declared by the manufacturer and that they can be achieved in a particular laboratory under normal conditions of routine operation.

The subject of verification is therefore not the IVD product itself, but the ability to perform the measurement process in a specific laboratory at a given time and space.

The verification should also result in a robust estimate of the measurement uncertainty. The minimum of the IVD MD product verification plan is:

- Precision (repeatability, intermediate precision).
- Bias (bias) quantified as recovery.
- Working range.

These features are necessary for a basic estimate of the uncertainty of the measurement results. The laboratory may verify other analytical characteristics if necessary.

10.3 Validation of modifications made to the originally validated method

An example is the use of an IVD MD test set/analytical measurement system where the procedure has been modified in some way. Such a modified test set should be subjected to 'cross' validation. This is done (according to [11]) by comparing the validation results of the original and the modified method. The aim is to document that the modification has not compromised the conformity of the properties with the requirements specified for the application.

10.4 Quantitative examinations developed by the laboratory (in-house)

Such a method must be validated in its entirety. This category includes methods developed in-house or adopted from another laboratory. In this case, a much broader and more comprehensive validation plan and investigation is necessary:

- Precision (repeatability, intermediate precision).
- Bias (bias), recovery.
- Working range, limit of detection and determination, linearity.
- Interference.
- Comparison with another method.
- Reference intervals and clinical decision limits.

The IUPAC Technical Report Harmonized Guidelines for Single-laboratory Validation of Methods of Analysis [15] deals in detail with validation from the position of a single laboratory and elaborates the laboratory approach in many variations.

11. Establishing a validation/verification plan

For both validation and verification, experimentally obtained values of the performance characteristics of the examination procedure (e.g. repeatability, intermediate precision and working range values) shall be compared with data supplied by the manufacturer in documentation of the measuring system or the originator of the analytical procedure or taken from data obtained during a previous validation. These predetermined criteria, together with the definition of the measurand and the selected acceptance characteristics of the procedure, form the core of the plan. Therefore, both validation and verification must follow a predefined plan that is approved by a staff member with appropriate authority and should include at least the following elements [8]:

1. the intended use of the laboratory examination procedure
2. the specification of the measurand
3. selection of relevant performance characteristics of the procedure
4. criteria for acceptance of the procedure as fit for purpose
5. the manner in which the experiments are to be performed
6. identification of the personnel responsible for performing the validation/verification, assessing the results and preparing and approving the report.

12. Implementation of the validation/verification plan

- Formulate the requirements to be achieved for the intended use (for IVD MD products, these requirements are part of the working documentation supplied by the manufacturer and the laboratory only verifies their validity).

- Determine the scope of the validation/verification, i.e. the selection of validation parameters - the basic criterion is to obtain sufficient data to assess whether the method is suitable for the intended purpose, develop a plan.
- Verify that the analytical instrumentation used is qualified for the purpose, i.e. that its technical parameters are sufficient for the purpose, that it has been properly calibrated, etc.
- Select the samples on which the experiments are to be carried out.
- Carry out the relevant experiments.
- Evaluate the results.
- If necessary, take measures to ensure that the specified requirements are met.
- Produce validation/verification documentation in the form of a report.

13. Performance characteristics of the laboratory examination procedure

The specific choice of which performance characteristics have to be examined and how to investigate them is a complex matter requiring expert judgement. In general, it is suggested [8] to use a risk management (i.e., professional judgment) to achieve the desired balance between meeting the specified requirements and obtaining statistically reliable data at reasonable cost to decide whether the parameter being investigated is consistent with the predefined criterion. For these reasons, this document, unlike the previous Recommendation, will not specify the number of samples required for each validation and/or verification experiment or prescribe the levels of any parameter. One simplistic recommendation cannot cover the requirements that the wide variety of laboratory examinations performed in medical laboratories require. It is advisable to consider carefully what the laboratory examination will be used for and to evaluate all the information available on the examination procedure.

The EN ISO 15189 standard [1] and reference [8] list the following parameters as performance characteristics of the laboratory examination procedure for validation and verification. Which ones are relevant for a given analytical procedure should be subject to expert judgement.

Table 1: Performance characteristics for analytical procedure validation/verification

Validation	Verification
• measurement trueness • measurement accuracy • measurement precision, including repeatability of measurement and intermediate precision of measurement • measurement uncertainty • analytical specificity including interfering substances • reference interval and clinical decision limits • analytical sensitivity • limit of detection and limit of quantification • measurement range • diagnostic specificity • diagnostic sensitivity	• measurement precision - repeatability • intermediate precision • measurement trueness - bias • working range, detection limit • stability • comparison with another method, if available • clinical decision limits • interferences • measurement uncertainty

If any particular performance characteristics are not applicable or reliably evaluable due to the nature of the examination procedure or the prevalence of the condition, this should be documented and justified.

The definition of individual performance parameters, the design, conduct and evaluation of validation and verification experiments are discussed in detail in the EURACHEM Guide [2] and in several other available documents [5, 6, 11, 15]. A pragmatic approach to ensure verification in a large clinical laboratory is described by Antonelli et al [7].

13.1 Aspects closely related to validation/verification

- Purpose of measurement.
- Definition of analyte and parameter.
- Pre-analytical procedures - sample collection, sample and patient identification, transport and sample handling. A number of EFLM working group recommendations for the pre-analytical phase are available -EFLM-WG-PRE [16].
- Summary of minimum quality requirements for the measuring instrument.
- Metrological traceability of the values of the measurement standards (calibrators). Calibration of measurements.
- If a laboratory adopts a method used elsewhere without sufficient information and data on its validation, verification alone is not sufficient and validation must be performed in the laboratory.
- When using more than one instrumentation system for the same measurand, appropriate verification for each individual instrumentation system must be performed.
- If a laboratory examination procedure is modified, the impact of the specific modification (e.g. reduction of sample volume in paediatrics) on the performance characteristics of the procedure should be considered and documented and the extent of additional validation/verification of the procedure should be proposed.
- Bias, possibly recovery. It is ideal to determine bias using matrix certified reference materials that have analyte content values obtained by reference measurement procedures (CRM and SRM). Such samples are often unavailable to the routine laboratory. In practice, it is necessary to choose a procedure where the laboratory compares its results with those of other laboratories using the same control materials. This comparison can be made using control materials previously used in EQA programmes. The bias value obtained under repeatability conditions is suitable for processing into an estimate of measurement uncertainty.
- Comparability of methods: The ideal comparison method is the reference measurement procedure. It is usually unavailable in routine practice. Therefore, the procedure is to compare the new routine method/procedure/analytical system with another routine method/procedure/analytical system that has been previously validated. If we are validating a POCT method/analytical system, then we use routine laboratory methods as comparators.
- Validation of the calculations used.
- Sensitivity, linearity, measuring range, limit of detection, limit of quantification.
- Robustness.
- Measurement uncertainty.
- Provision of a minimum quality management programme and its limit parameters.
- Complete quality control documentation.

All these items must form an integrated, continuously designed and monitored system in the laboratory, as described in ISO 15189 [1]. Solving partial problems without linking them to the others is irrelevant.

14. Documentation

The validation/verification documentation in the laboratory shall consist of the validation/verification plan, the results obtained including raw data, and a validation/verification report containing a statement as to whether the method is suitable for the intended use. The results obtained must be demonstrably compared with the acceptance criteria specified in the validation/verification plan. The report must be assessed and authorized by a staff member with the appropriate authority. The results and report must be stored for at least the period of use of the laboratory examination procedure. The appropriate format of the plan and report is described in the Appendix to Eurachem Guide [3].

15. Release and implementation

A SOP and appropriate internal and external quality control procedures with health significance thresholds must be developed for a laboratory examination procedure that has been successfully validated or verified. Release and implementation must be carried out according to the general principles applicable in the laboratory. If an examination procedure is being re-established after a change has been made, it is appropriate to inform the requester of the change, particularly where this might have consequences for the interpretation of the results.

16. Summary of validation principles

The laboratory must validate laboratory examination procedures from the following sources:

a) non-standard and non-harmonised methods;
b) methods designed or developed by the laboratory;
c) standard methods used outside their intended scope of use;
d) validated methods that have been subsequently modified.

For this purpose:

- Determine and document validation parameters and analytical characteristics (manufacturers' data on analytical characteristics of the method/measurement system).
- Determine and document the relevant acceptance criteria (analytical characteristic values specifying the requirements for the measurement system to meet the intended use).
- Plan validation experiments.
- Prior to the start of the experiments, make sufficiently familiar with the instrument and method, detailed training of personnel, and verification and calibration of instrumentation and computer technology, including statistical programs.
- Carry out the experiments.
- Reassess the criteria and repeat the experiments if necessary.
- Summarise and evaluate the results, produce a validation report.
- Propose a quality assurance and quality control procedures.
- Produce appropriate SOPs.
- Develop a revalidation plan and define criteria for this.

17. Summary of verification principles

Validated laboratory examination procedures used without modification shall be subject to independent verification under laboratory conditions before routine use is initiated.

For this purpose:

- Select and document the key characteristics of the analytical method to be verified (the laboratory must obtain information from the manufacturer/ originator of the method to confirm the performance characteristics of the procedure) and establish the required values for the performance characteristics of the method.
- Plan experiments for verification.
- Ensure that personnel are sufficiently familiar with the instrument and method, and that instrumentation and computer equipment, including statistical programs, are checked and calibrated before experiments begin.
- Perform the experiments.
- Summarise and evaluate the results, prepare a verification report.
- Propose a quality control method.
- Produce the relevant SOPs.
- Develop a plan for periodic repetition of verification and define criteria for this.

18. References

1. ISO 15189:2012 ed. 2. Medical laboratories - Requirements for quality and competence. ISO, Geneva.
2. EURACHEM GUIDE The Fitness for Purpose of Analytical Methods. Laboratory guide to method validation and related topics. Editors B. Magnusson, U. Örnemark. Second edition. Available from www.eurachem.org
3. Planning and reporting of method validation studies - Supplement to the Eurachem Guide on the Fitness for Purpose of Analytical Methods (2019). V. Barwick (Ed.), Available at http://www.eurachem.org.
4. M. L. J. Weitzel, S. M. Lee, M. Smoot, N. Viafara, M. Brodsky How to meet ISO 17025 requirements for method verification. AOAC International, (2007) Available at: https://members.aoac.org/AOAC_Docs/lptp/alacc_guide_2008.pdf
5. Theodorsson E, Magnusson B.: Full method validation in clinical chemistry. Accred Qual Assur 2017, 22:235-246.
6. General Accreditation Guidelines - Validation and Verification of Quantitative and Qualitative Test Methods NATA 2018. Available at https://www.nata.com.au/phocadownload/gen-accreditation-guidance/Validation-and-Verification-of-Quantitative-and-Qualitative-Test-Methods.pdf.
7. Antonelli G, Padoan A, Aita A, Sciacovelli L, Plebani M. Verification of testing procedures in the clinical laboratory for imprecision, trueness and diagnostic accuracy according to ISO15189:2012: a pragmatic approach. Clin Chem Lab Med 2017, 55:1501-1508.
8. Roelofsen-de Beer R, Wielders J, Boursier G, Vodnik T, Vanstapel F, et al: Validation and verification procedures in medical laboratories: position paper of the EFLM Working Group on Acceptance and ISO/CEN standards (WG-A/ISO) on deasling with the requirements of ISO 15189:2012 on method verification and validation. Clin Chem Lab Med 2019, 57/3:361-367.
9. ISO 9000: 2015 Quality management systems - Fundamentals and vocabulary. ISO, Geneva.
10. JCGM 200:2012 International vocabulary of metrology – Basic and general concepts and associated terms (VIM) 3rd edition. Available from www.bipm.org
11. EN 13612:2002. Performance evaluation of in vitro diagnostic medical devices. CEN 2002.
12. Guidelines for the validation of bioanalytical methods for industry. FDA 2018. Available at https://www.fda.gov/media/70858/download.
13. ISO 15198:2004. Clinical laboratory medicine - In vitro diagnostic medical devices - Validation of user quality control procedures by the manufacturer. ISO Geneva.
14. ISO 13485 ed. 2 :2016. Medical devices - Quality management systems - Requirements for regulatory purposes. ISO, Geneva.
15. Thompson M, Ellison SLR, Wood R.: Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report) Pure and Appl. Chem 2002, 74/5:835-855. (Harmonized guidelines for single-laboratory validation of methods of analysis).
16. Vermeesch P, Frans G, von Meyer A, Costelloe S, Lippi G et al: How to meet the preanalytical requirements of ISO 15189:2012 in clinical laboratories? Clin Chem Lab Med 2021, DOI:10.1515/cclm-2020-1859.