



Verification or validation, that is the question

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Abstract: The latest revision of ISO 15189 standard in 2012 is now universally considered as the most important document for accreditation of medical laboratories. This standard is aimed to verify and validate examination procedures (EPs). Verification is “provision of objective evidence that a given item fulfils specified requirements”, while validation is “verification, where the specified requirements are adequate for the intended use”. When used without modification, validated EPs shall be verified, whilst non-standard methods, home brew methods, validated methods which have been modified or are being used outside their intended scope shall be validated before being introduced into clinical and laboratory practice. In order to fulfil the above requirements, three different approaches were used for: (I) verification of EPs already in use in laboratory; (II) verification of newly introduced EPs; (III) validation of EPs. For verification, at least two performance characteristics should be verified according to the intended use of specific EPs. Different operative flows are described for quantitative, qualitative and semi-quantitative methods. For validation procedure, a validation plan should be planned, including performance characteristics for specific EP, the experimental procedure and acceptability criterion. The final step of the validation process is production of a validation certificate. This is aimed to summarizing the validation process and defining the approval for being used in clinical practice. The proposed verification and validation procedures were actually fulfilling the ISO 15189 requirements during the accreditation audit. They can hence represent a model to follow for other medical laboratories.

Keywords: ISO 15189; verification; validation; examination procedure (EP); accreditation

Received: 19 July 2017; Accepted: 20 July 2017; Published: 11 August 2017.

doi: 10.21037/jlpm.2017.07.11

View this article at: <http://dx.doi.org/10.21037/jlpm.2017.07.11>

ISO 15189:2012 requirements 5.5.1.2 and 5.5.1.3

The latest revision of ISO 15189 standard in 2012 is now universally considered the most important document for accreditation of medical laboratories (1), and 60 countries have made it mandatory for clinical laboratories so far (2). The standard focuses on patient care as the main objective of laboratory services, which can be achieved by verifying management organization, responsibilities and technical quality, and by adopting the philosophy of continuous improvement (3). The technical needs focus on mitigating laboratory errors through evaluation of the total testing process. Regarding the analytical phase, this standard entails verification and validation of examination procedures (EPs)

(section 5.5.1.2 and 5.5.1.3). According to International Vocabulary of Metrology 3 (VIM 3), the definition of verification is “provision of objective evidence that a given item fulfils specified requirements” (4). In particular, the ISO 15189 states that “*the independent verification by the laboratory shall confirm, through obtaining objective evidence (in the form of performance characteristics) that the performance claims for the EP have been met. The performance claims for the EP confirmed during the verification process shall be those relevant to the intended use of examination results.*” (1). Validation is defined as “verification, where the specified requirements are adequate for the intended use” (4). In particular, ISO 15189 attests that “*the validation shall be as extensive as is necessary and confirm, through the provision of*

Table 1 Scheme of the procedure for the verification of examination procedures already in use by at least 2 years

A. Quantitative EP
A.1 Imprecision verification (3 operative flow charts)
A.1.1 When the manufacturer declares the imprecision and the IQC are >20/year
A.1.2 When the manufacturer doesn't declare the imprecision and the IQC are >20/year
A.1.3 When the IQC are <20/year or are not available
A.2 Trueness verification (4 operative flow charts)
A.2.1 When EQAS are available and the manufacturer doesn't claim the trueness
A.2.2 When EQAS are available and the manufacturer claims the trueness
A.2.3 When EQAS are not available and the manufacturer claims the trueness
A.2.4 When EQAS are not available and the manufacturer doesn't claim the trueness
B. Qualitative EP
B.1 Diagnostic accuracy (Se and Sp) verification (2 operative flow charts)
B.1.1 When the manufacturer declares the diagnostic accuracy
B.1.2 When the manufacturer doesn't declare the diagnostic accuracy
C. Semi-quantitative EP
C.1 Imprecision verification ⇒ follow the operative flow charts described for the quantitative EP
C.2 Diagnostic accuracy (Se and Sp) verification ⇒ follow the operative flow charts described for the qualitative EP

The detailed description is reported elsewhere (7). EP, examination procedure; IQC, internal quality control; EQAS, external quality assurance scheme; Se, diagnostic sensitivity; Sp, diagnostic specificity.

objective evidence (in the form of performance characteristics), that the specific requirements for the intended use of examination have been fulfilled." (1).

When used without modification, the validated EP shall be verified, whilst non-standard methods, home brew methods, validated methods which have been modified or are being used outside their intended scope shall be validated. The main objective of validation of an EP is to demonstrate its fitness-for-purpose (5).

The pragmatic approach to verify and validate the examination procedures (EPs)

In order to fulfil the above requirements, three different approaches were used for:

- (I) Verification of EPs already in use in laboratory;
- (II) Verification of newly introduced EPs;
- (III) Validation of EPs.

Verification of EPs already in use in laboratory

The developed operative flow for EPs already in use for over

two years is based on consideration that the accreditation process is developed in a laboratory where the quality system has already been successfully implemented (6,7). Therefore, the operative procedures refer to a laboratory in which the total testing process is under control, users' satisfaction is monitored and an improvement process is ongoing. Therefore, if the EP provides results satisfying clinical needs and, subsequently, the purpose of the test, the verification can be limited to parameters focused on the accuracy of test results. This approach is described in detail elsewhere (8), and summarized in *Table 1*.

Verification of newly introduced EPs

Following the same approach as for verification of EPs already in use, different procedures were identified for quantitative, qualitative and semi-quantitative newly-introduced EPs.

Verification of newly introduced quantitative EPs

For quantitative EPs, the document CLSI EP15-A3 (9) was analysed for establishing the most suitable workflow. As for

quantitative EPs already used in the laboratory, no less than imprecision and trueness need to be verified in terms of CV% and bias%, respectively.

Imprecision verification

As suggested by CLSI EP15-A3 (9), the imprecision verification study consisted of three parts, as summarized in *Figure 1*.

- (I) Repeated measurements over 5 days of at least two patients' samples. For this study, patient samples, pool of patient samples or commercially available quality controls can be used, in this hierarchical order of priority. The samples should have different concentrations and in particular, the concentrations should be close to those validated by the manufacturer. The proposed basic design (3×5) needs five days, three replicates per run, one run per day, for a total of 15 results per sample. In relation to the measurand stability, the length of the design can be reduced to a 3×4 scheme, but not less than a 3×3 design should be used. The presence of outliers needs to be investigated using the Grubbs' test. If more than one outlier is observed throughout the entire study, the verification of imprecision study should then be repeated.
- (II) Calculations of within-laboratory imprecision (S_{WL}) and laboratory repeatability (intra-assay- S_R).
- (III) Assessment of uniformity with claims and acceptability of test results. For each level, the S_{WL} should be less than the total imprecision claimed by the manufacturer (σ_{WL}). When S_{WL} is higher than σ_{WL} , the upper verification limit (UVL_{AVL}) needs to be calculated, as described in the CLSI EP15-A3 (9), by considering the degrees of freedom and the UVL factor. If the S_{WL} is higher than the respective UVL_{AVL} , a clinically allowable limit of variability ($CV\%_{ref}$) needs to be defined. This limit can be identified following the hierarchical structure established in the 1999 Stockholm Consensus Conference (10) and revised in the 1st strategic conference held in Milan (11), according to: (i) clinical recommendations; (ii) biological variation; (iii) state-of-the-art, as we proposed for verification of EPs already used in the laboratory (8). If S_{WL} is higher than $CV\%_{ref}$, the S_R has to be compared with the intra-assay imprecision claimed by manufacturer (σ_R). If S_R is higher than σ_R , the UVL_R needs to be calculated, as previously discussed. If the S_R is higher than the respective UVL_R , the

verification process fails.

Trueness verification

Trueness is estimated by analyzing materials with known concentration, comparing results with target values, and establishing the verification interval, as described in the CLSI EP15-A3 document (9). The materials with known concentration can be: (I) materials whose concentrations can be adjusted to the desired levels, with negligible imprecision, e.g., by spiking a therapeutic drug into patient sample pools known to be analyte-free; (II) certified reference standards; (III) survey materials from PT/EQA programs; (IV) materials used in inter-laboratory quality control programs; (V) materials intended for routine internal quality control with formerly assigned target values. The mean value obtained in these studies should be included in the verification interval. If the verification interval did not include the mean value, the bias should be calculated ($Bias\%_{LAB}$). If the manufacturer provides a declared bias, the bias obtained by the laboratory should be less than or equal to that claimed by manufacturer. If the manufacturer did not declare this value, a specific allowable bias ($Bias\%_{ref}$) has to be identified, according to criteria reported for imprecision. If the $Bias\%_{LAB}$ is higher than the $Bias\%_{ref}$, then the verification fails. When materials at known concentrations are unavailable, another performance characteristic should be verified according to performances claimed by the manufacturer, so that at least two performance characteristics are verified.

Verification of newly introduced qualitative EPs

For qualitative EPs, the document CLSI EP12 (12) is used as a guideline. For this type of procedures, the diagnostic accuracy is evaluated, in terms of sensitivity and specificity, using not less than ten true negative samples and not less than ten true positive patient samples. To verify this parameter, the manufacturer needs to declare the diagnostic accuracy in terms of diagnostic sensitivity, diagnostic specificity, number of true positive and true negative samples. The 95% confidence intervals (95% CI) are calculated with the Wilson's method, as we proposed for verification of EPs already used in laboratory (8). The diagnostic sensitivity and specificity obtained by the local laboratory should be included in the calculated 95% CI derived from manufacturer's data.

Verification of newly introduced semi-quantitative EPs

For semi-quantitative EPs, imprecision and diagnostic

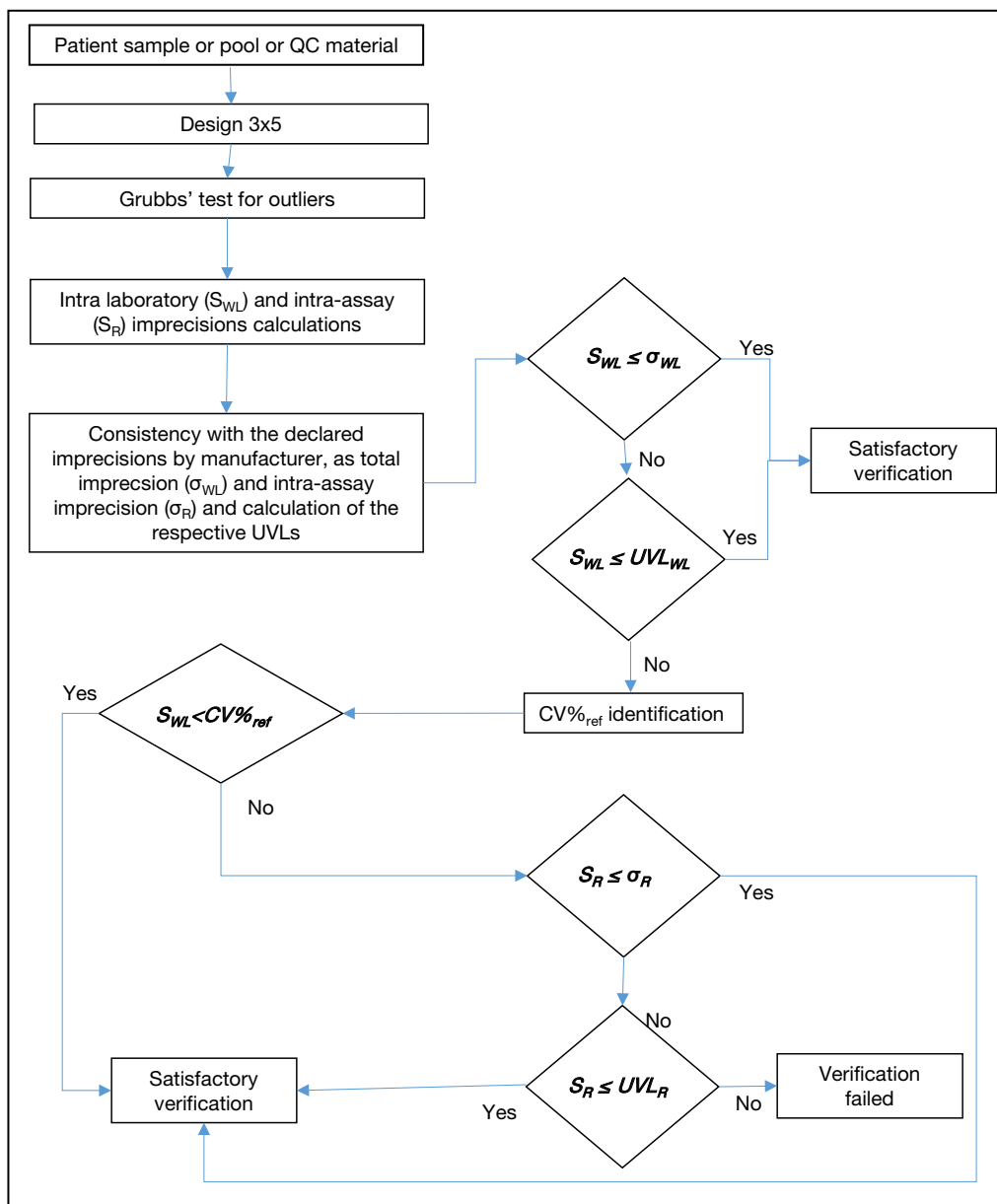


Figure 1 Operative flow for imprecision verification of newly introduced examination procedures. S_{WL} , within-laboratory imprecision; S_R , laboratory repeatability; σ_{WL} , manufacturer total imprecision; σ_R , manufacturer intra-assay imprecision; UVLs, upper limits of verification; UVL_{WL} , upper limit of verification for total imprecision; UVL_R , upper limit of verification for intra-assay imprecision; $CV\%_{ref}$, clinically allowable limit of variability.

accuracy are assessed, as described above for quantitative and qualitative EPs, respectively.

Validation of EPs

The validation process consists of six parts:

- (I) Analysis of available scientific documentation

pertinent to specific EP. In particular, clinical guidelines, recommendations and peer-reviewed texts or journals should be considered. In our experience, documents are selected for validation of mass spectrometry-based methods (13,14), for chromatographic techniques (15,16), for ligand-binding assays (15), for cell-based fluorescence

Date: __/__/__

VALIDATION PLAN

Examination procedure for the analysis of _____

Analytical method: _____

Intended use: _____

Performance characteristic	Typology of test	Executive time	Acceptability criteria	Related document

Signature: _____

Figure 2 Template of a validation plan.

- assay (17), and for multiplex nucleic acid assays (18).
- (II) Evaluation of the intended use of EP.
- (III) Identification of performance characteristics for specific EP. The choice of the performance characteristics is strictly dependent on the necessity to confirm that specific requirements for intended use are fulfilled, as mandated by the ISO 15189 (1).
- (IV) Definition of experimental procedure: the type of the tests to be performed, thus including number of samples and replicates, along with the time need for testing should be determined.
- (V) Identification of acceptability criterion for evaluating results according to the appropriateness of intended use. The acceptability of results should be evaluated according to reliable scientific documents, previously identified.

A validation plan should be planned, summarizing all these points. The template of a validation plan is briefly shown in *Figure 2*. All results obtained during the validation process should be recorded, along with instrumental output. In fact, the ISO 15189 states that “*the laboratory shall document the procedure used for the validation and record the results obtained*” (1).

- (VI) Production of a validation certificate, i.e., the

final step of the validation process. The validation certificate is a tool aimed to summarize the validation process and define the approval for use in clinical practice. The validation certificate should report type of EP, intended use of the test, reference documents, results obtained during the evaluation of performance characteristics and approval for use. In particular, the personnel who carried out the validation and who is responsible for specific EP has the duty of the fitness for purpose of validating EP. The Laboratory Director has the responsibility for adopting and using the EP in clinical practice. The template of a validation certificate is shown in *Figure 3*.

Conclusions

The International Standard ISO 15189 is widely recognized as the guideline for medical laboratories accreditation. Despite the first edition was published in 2007, this guideline has not been extensively implemented so far by medical laboratories at an international level, with large variations between different Countries and Regions. For example, there are only six accredited laboratories in Italy (19). The verification and validation of the EPs are novel

VALIDATION CERTIFICATE			
Examination procedure for the analysis of _____			
Analytical method: _____			
Intended use: _____			
Description of the analytical procedure: _____			
References: _____			
Performance characteristic	Obtained results	Acceptability	
Approval to the use in the clinical practice			
Responsible	Name and Surname	Signature for appropriateness to the intended use	Date
<i>Responsible for the validation process</i>			
<i>Responsible for the specific EP</i>			
	Name and Surname	Signature for appropriateness to the use in the clinical practice	Date
<i>Laboratory Director</i>			

Figure 3 Template of a validation certificate.

requirements compared to the more widespread ISO 9001 certification. The development of operating procedures based on a practice approach should help medical laboratories implementing the accreditation process, thus considering the balance between technological possibilities, risks and costs. The proposed verification and validation procedures have been recognized to comply with the ISO 15189 requirements during the accreditation audit. Therefore they should represent a valuable model to be followed by other medical laboratories.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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doi: 10.21037/jlpm.2017.07.11

Cite this article as: Antonelli G, Padoan A, Aita A, Sciacovelli L, Plebani M. Verification or validation, that is the question. *J Lab Precis Med* 2017;2:58.