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# Procedures for Validation of Diagnostic Methods in Clinical Laboratory Accredited by ISO 15189

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## 1. Introduction

Actually, each clinical and/or biochemical laboratory has responsibility for demonstrating its competence and therefore must obtain results of good quality. Medical laboratories provide vital medical services to different clients: clinicians requesting a test, patients from whom the sample was collected, public health and medical-legal instances, referral laboratories and authoritative bodies. All expect results that are accurate and obtained in an effective manner, within a suitable time frame and at acceptable cost. There are different ways of achieving the end results, but compliance with International Organization for Standardization (ISO) 15189, the international standard for the accreditation of medical laboratories, is becoming progressively accepted as the optimal approach to assuring quality in medical testing. As result, the accreditation of clinical laboratories is shifting from being a “recommendation” to becoming a “requirement” in many countries throughout Europe and in the other countries around the world (Berwouts, 2010). *Accreditation* is defined by ISO as the “Procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks”. Although accreditation also considers the quality management system (QMS), it has additional formal requirements of technical competence, including initial and continuous training of personnel, *validation of methods and instruments*, and internal and external quality control.

A good QMS in the laboratory has a lot of advantages such as increased transparency, traceability, uniformity, work satisfaction and better focus on critical points. On the contrary, it will require extra time on aspects such as document control and there is a danger of losing critical attitude and curbing innovation and changes. Therefore, a formal accreditation and the linked periodical audits are stimulant for keeping the quality system (QS) alive. Without accreditation, there is a danger of giving less attention to quality improvement. In addition, accreditation is a good way to demonstrate and attest competence and a worldwide tool to recognize laboratories. Finally, all parties (patients, families, the laboratory and clinicians) are benefited through better processes and quality of results (Berwouts, 2010).

All essential elements of QS are covered by the ISO 15189 accreditation standard in two distinct chapters: management requirements and technical requirements. Technical elements

enclose personnel and training, accommodation, equipment, *validation* and assuring quality of examination procedures by internal quality control (IQC), external quality control (EQA), maintenance and calibration.

ISO 15189 standard emphasizes so in the quality of contributions to patient care as in laboratory and management procedures and specifies the quality management system requirements, in particular to medical laboratories and stages:

<<The laboratory shall use only validated procedures for confirming that the examination procedures are suitable for intended use>>, <<The validation shall be as extensive as are necessary to meet the needs in the given application or field of application>>, and <<Procedures need to be periodically revalidated in view of changing conditions and technical advances>>.

IQC is an internal verification that the test yields consistent results day after day; in the other words, the identification measure of *precision*, but not necessarily of *accuracy*. ISO 15189 requires that “the laboratory shall design IQC systems that verify the attainment of the intended quality of results”. On the hand, the laboratory should avoid mistakes (ISO 15189, 5.6.1.) in the process of handling samples, requests, examinations, reports and so on; on the other, the laboratory should determine *uncertainty* (ISO 15189, 5.6.2) where relevant and possible. For each test, the laboratory should identify and define potential errors, risks and challenges (typically, during the validation phase); subsequently, specific IQC should be defined to assure each risk and potential problem.

EQA is an important complement to IQC in which a large number of laboratories are provided with the same material and required to return results to a coordinating centre. The results are compared to determine the *accuracy* of the individual laboratory. In addition, EQA provides continuous education and training for laboratories as well. Accredited laboratories are required to “participate in interlaboratory comparisons such as those organized by EQA schemes” (ISO 15189, 5.6.4). EQA should, as far as possible, cover the entire range of tests, and the entire examination process, from sample reception, preparation and analysis to interpretation and reporting (ISO 15189 5.6.5). For some specific tests, no EQA scheme exists. ISO 15189 (5.6.5) states “whenever a formal laboratory comparison programme is not available, the laboratory shall develop a mechanism for determining the acceptability of procedures nor otherwise evaluated”; examples include reference materials or interlaboratory exchange. Interlaboratory comparisons should cover the scope of services offered and there should be a formal mechanism of review and comparison of results.

Used together, IQC and EQA provide a method of ensuring accuracy and consistency of results and are vital tools in the laboratory. The relation between precision and accuracy may be illustrated by the familiar example of shooting arrows at a target (Berwouts, 2010; Burnett, 2006) (figure 1).

The results provided by the clinical/medical laboratory must be accurate to allow a correct clinical interpretation and to be comparable with earlier or later and between laboratories. So the purpose of this chapter is to establish a set of guidelines and recommendations to help personnel carry out their work in clinical/medical laboratories that are accredited or under accreditation by ISO 15189. It is necessary to establish and define the different procedures validation, the fundamental guidelines for the proper design of the validation, the recommendations to validate an established method in the laboratory, and the different parameters to be assessed.

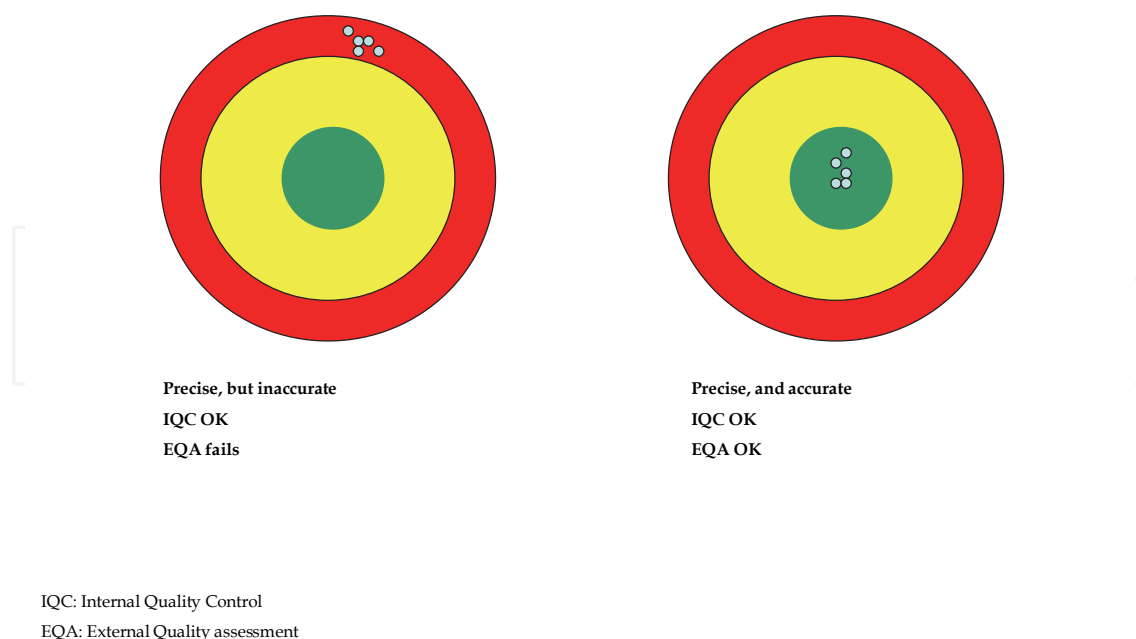


Fig. 1. Accuracy and precision.

## 2. Validation design of a method

*Diagnostic validation* is a formal requirement of accreditation standards, including ISO 17025 and ISO 15189, those tests/methods and instruments must be validated before diagnostic use to ensure reliable results for patients, clinicians or referring laboratories and their quality must be maintained throughout use. In other words, the laboratory must demonstrate that their tests/methods are fit for the intended use before application to patient samples. Figure 2 shows a summary of what ISO 15189 states with regard to validation (Berwouts, 2010; Burnett & C. Blair, 2001, Burnett et al., 2002; Burnett, 2006).

Although the concept of validation makes explicit reference to the purely analytical aspects may also include preanalytical and sampling procedures, handling and transport. At a minimum, the techniques used to determine the performance of a method should be one or more of the following:

- Calibration using reference standards or reference materials or traceable to these
- Comparison of results obtained with other methods
- Interlaboratory comparisons
- Systematic evaluation of the factors that influence outcomes
- Estimate of the uncertainty of the results based on scientific knowledge of theoretical principles of the method and practical experience.

In any case, analytical methods must be those that meet customer requirements, that is, those that provide clinically useful information. Thus, an analytical method for determination of aluminium in serum based on the complexation of this element with 8-hydroxyquinoline and quantification by fluorimetry can have high reliability but its detection limit is at least an order of magnitude above the upper limit of the reference element, which makes this method, does not meet customer requirements.

There are publications that provide general methods of evaluation of analytical methods including the following:

1. Definition of the evaluation protocol that registers the results of the measurements.
2. Determining the range of application and dilution mechanisms, if any.
3. Identify the components of precision in the day, every day.
4. To determine the accuracy through recovery studies before a definitive method or reference, if any.
5. To determine the sensitivity.
6. Estimate the limit of detection and quantification (and others if applicable).
7. To study the specificity of the method, checking for interference.
8. Establishing the reference range.
9. Document the validity of the method.

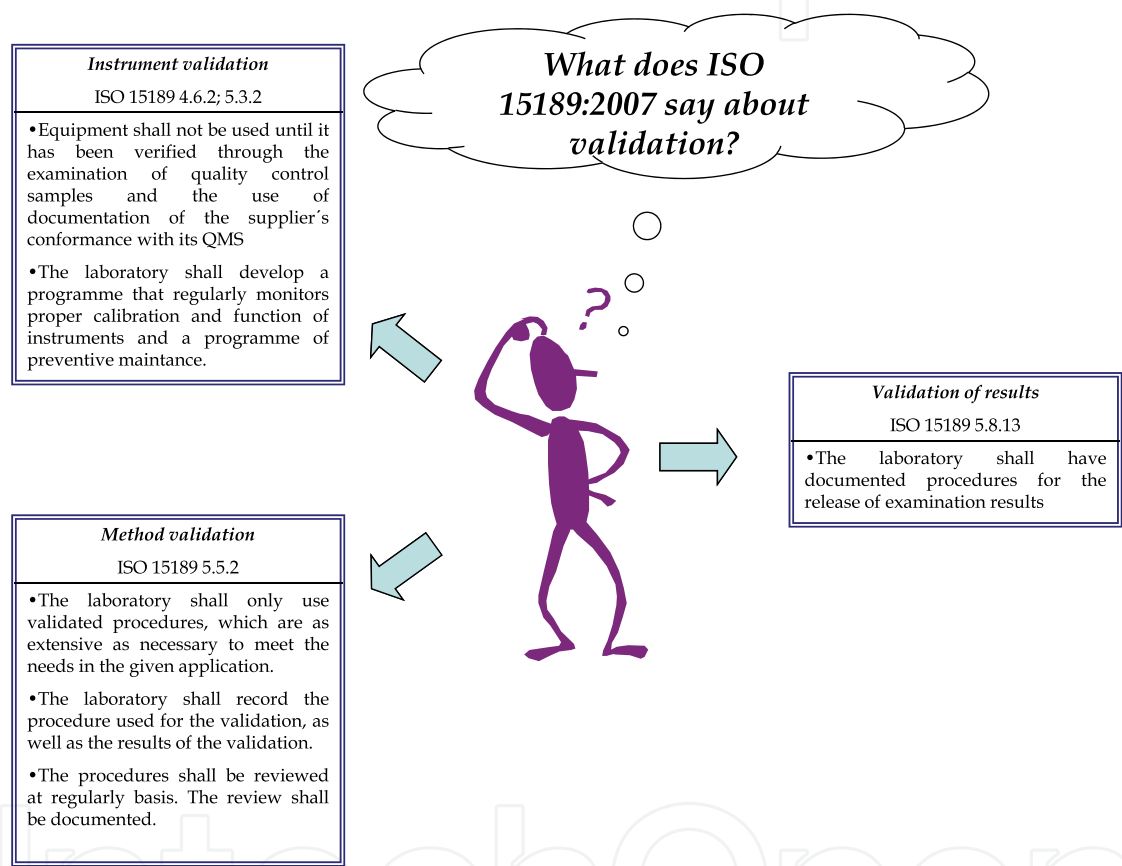


Fig. 2. Validation requirements according ISO 15189:2007.

Regarding the validation and control of analytical procedures, paragraph 5.5 of ISO 15189: 2007 specifies to be used those procedures "that have been published by experts or international guidelines, national or regional". Own procedures "must be properly validated for its intended use and fully documented".

So, ISO 15189: 2007 says "as appropriate, the documentation should include the following: technical specifications (e.g., linearity, precision, accuracy, expressed as a measurement uncertainty limit of detection, measurement, sensitivity and specificity, and interference)" (ISO 15189, 5.5.3).

When a unit or section of a clinical/medical laboratory chooses to engage in the accreditation ISO 15189, must be aware that although the analytical methods which usually works have been validated in its implementation must be validated in time.

A validation process like any other requires a series of planning, execution and control to ensure that the results come to fruition.

a. Planning:

- Definition responsible for performing the validation process.
- Definition of objectives and internal requirements applied the method to validate (purpose, parameters to measure in the matrix or matrices to be determined).
- Definition and documentation of the method (procedure for validation).

b. Implementation: implementation of activities, results obtained and recorded (date, operators and results).

c. Control:

- Verification of compliance with targets.
- Final Declaration of the appropriateness of the procedure defined.

a. *Planning* consists of the following phases:

1) Assign responsibility. In this phase it will be defined a person responsible for carrying out the validation process and deciding the outcome. This person can count on help from others, but he is responsible for making decisions so, he must have a proper qualification; 2) Definition of the characteristics and requirements applied to the method: The definition of requirements has to do with the intended use of the method ( i.e. as property or analyte, the matrix or matrices in which they will determine the use that will make the test results and legal requirements or economic policy to be applied to test results), from the specified requirements and based on a literature search using other standards, etc. There is a design and optimization phase of the procedure that is performed by laboratory. This is the stage where, for example for an instrumental method, you must establish a priori the linearity of the method, the working range, the limit of detection and quantification is desired, the accuracy and precision fit. In short what features the laboratory can apply the method to the intended use; 3) Description documented procedure: It should be sufficiently detailed to ensure its proper performance and repeatability. This ensures that all laboratory personnel that are qualified can do just as the method with comparable results.

To accomplish this phase can be helpful in establishing a suitable index of the case as the reference standard.

b. *Implementation*: Outcome is based on the realization of a series of tests and experiments that occur as a result values for the parameters defined in the requirements. These parameters can be variable depending on the type of method applied and the requirements and can include accuracy, precision, limit of detection, limit of quantization, selectivity, etc.

c. *Control*: The control is the verification of compliance and the final declaration. 1) Verification of compliance: As a result of the implementation of activities will be decided whether the values meet the specified requirements, in which case proceed to establish which checks should be made to the method as regular monitoring to confirm that remain requirements requested at the time of validation, e.g. using a control pattern periodically check the parameters of the regression line, etc., proceeding to their inclusion in the proceedings and preparing a final edition of the same. Otherwise you may be assessed if an amendment to the previously established requirements. 2) Final Statement: All the validation process should conclude with a formal statement of the adequacy of the procedure defined as stated is suitable for their intended use, according to specified requirements (Burnett & C. Blair, 2001; Burnett et al., 2001; Burnett, 2006).



2.1 Types and methods of validation

The laboratory shall validate examination procedures from non standard methods, laboratory designed or development methods, standard methods used outside their intended scope and modified validated methods.

When examination procedures have been validated by the method developer (i.e., the manufacturer or author of a published procedure), the laboratory shall obtain information from the method developer to confirm that the performance characteristics of the method are appropriate for its intended use. When changes are made to a validated examination procedure, the influence of such changes shall be documented and, if appropriate, a new validation shall be carried out.

Examination procedures from method developers that used without modification shall be subject to verification before being introduced into routine use. The verification shall confirm, through provision of objective evidence (performance characteristics), that the performance claims for the examination method have been met. The performance claims for the examination method confirmed during the verification process shall be those relevant to the intended use of the examination results.

Verification and validation are two slightly different procedures (figure 3). By default, all new laboratory procedures must be validated before application to clinical testing. In addition, a validation is necessary when major technical modifications to existing methods are carried out or when the performance of existing methods has been shown to be unsatisfactory (Berwouts, 2010; Hauck et al., 2008).

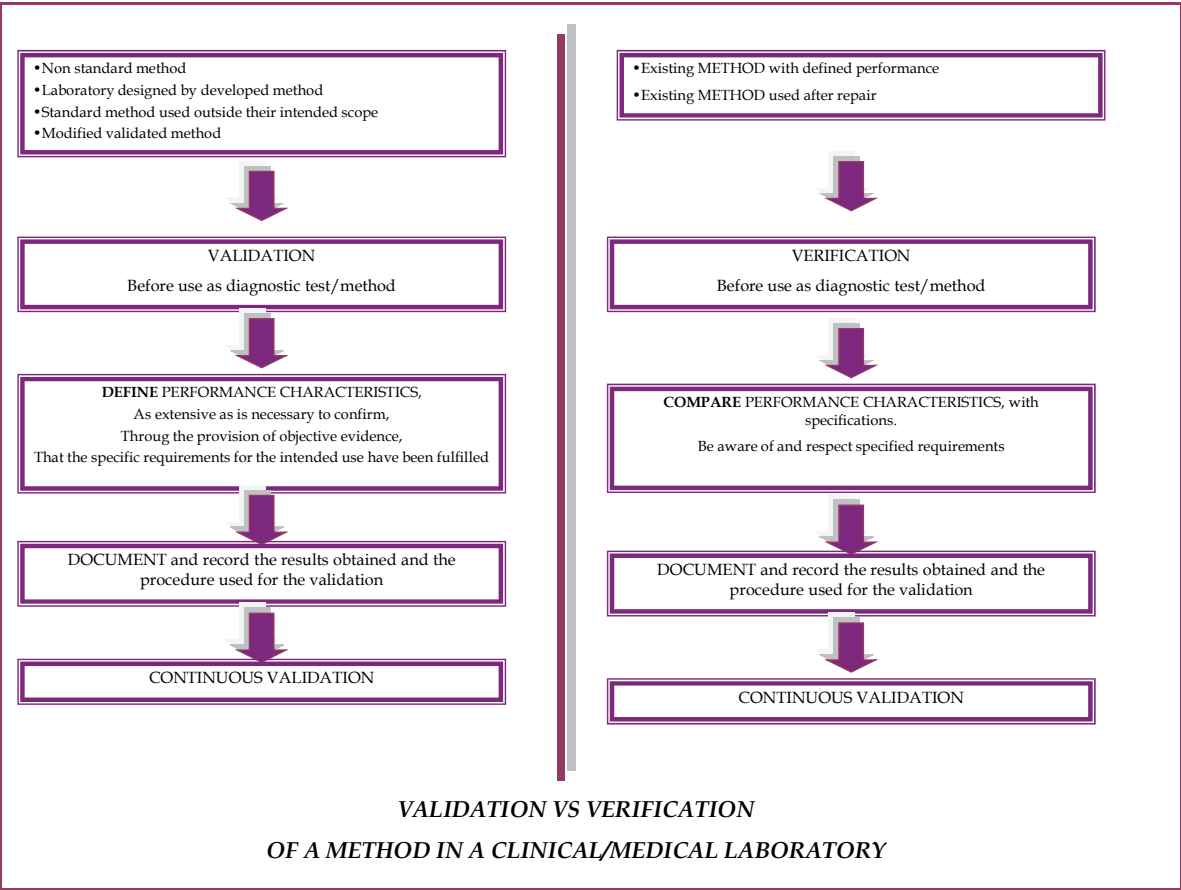


Fig. 3. Validation vs verification in diagnostic methods.

The validation or verification of methods, as defined in figure 4, is a normal requirement for the accreditation of laboratories according to the two major international standards applicable to clinical/medical laboratories, ISO 15189 and ISO 17025. Although the general requirements are clearly stablished (figure 4), the standards provide very little guidance about the detailed requirements or procedures.

Before a test/method can be validated, it is necessary to establish (a) that the particular measurements are diagnostically useful and (b) that the correct analyte(s), and only the correct analyte(s), are measured.

Full validation is required when is no suitable performance specification available, for example, with novel tests/methods or technologies. This process involves assessing the performance of the test/method in comparison with a “gold standard” or reference test/method that is capable of assigning the sample status without error. In simple terms, validation can be seen as a process to determine whether the laboratory is “performing the correct test/method”. Validation data can be used to assess the accuracy of either the technology or the specific test/method. Generally speaking, the generic validation of a novel technology should be performed on a larger scale, ideally in multiple laboratories (interlaboratory validation), and should include a much more comprehensive investigation of the critical parameters relevant to the specific technology to provide the highest chance of detecting sources of variation and interference (Berwouts, 2010; Burnett, 2006).

Principle requirements of ISO 157025:2005	Principle requirements od ISO 15189:2007
<p>5.4.2 “Laboratory-developed methods or methods adopted by the laboratory may also be used if they are appropriate for the intended use and if they are validated”.</p> <p>5.4.5.2 “The laboratory shall validate non-standard methods, laboratory-designed/developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods to confirm that methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application or field or application.The laboratory shall record the results obtained, the procedure used for validation, and a statement as to wether the method is fit for the intended use”.</p> <p>5.4.5.3 “NOTE 1 validation includes specification of the requirements, determination of the characteristics of the methods, a check that the requirements can be fulfilled by using the method, and a statement on the validity. NOTE 3 Validation is always a balance between costs, risks and technical possibilities, There are many cases in which the range and uncertainty of the values (eg accuracy, detection limit, selectivity, linearity, repeatability, robutness and cross-sensivity) can only be given in a simplified way due to lack of information”.</p>	<p>5.5.1 “[...]If in-house procedures are used, they shall be appropriately validated for their intended use and fully documented”.</p> <p>5.5.2 “The methods and procedures selected for use shall be evaluated and found to give satisfactory results before used for medical examinations. A review of procedures by the laboratory director or designated person shall be undertaken initially and at defined intervals”.</p> <p>5.6.2 “The laboratory shall determine the uncertainty of results, where relevant and possible”.</p>

Fig. 4. Principle requirements of ISO 15189: 2007 and ISO 17025:2005 about validation and verification.



## 2.2 Recommendations to validate a method developed in the laboratory

### 2.2.1 Quantitative methods

Two components of analytical accuracy are required to characterize a quantitative method: trueness and precision. Trueness expresses how close the methods result is to the reference value. Typically, multiple measurements are made for each point and the rest method result is taken to be the mean of the replicate results (excluding outliers if necessary). As quantitative assays measure a continuous variable, mean results are often represented by a regression of data (a regression line is a linear average). Any deviation of this regression from the reference indicates a systematic error, which expressed as a bias (i.e., a number indicating the size and direction of the deviation from the true result). There are two general forms of bias. With constant bias, method results deviate from the reference value by the same amount, regardless of that value. With proportional bias, the deviation is proportional to the reference value. Both forms of bias can exist simultaneously. Although measurement of bias is useful, it is only one component of the measurement uncertainty and gives no indication of how dispersed the replicate results are. This dispersal is called precision and can be measured by imprecision, that provides an indication of how well a single method results is representative of a number of replicates or repetitions. Imprecision is commonly expressed as the standard deviation of the replicate results, but is often more informative to describe a confidence interval (CI) around the mean result. Precision is subdivided according to how replicate analyses are handled and evaluated.

Repeatability refers to the closeness of agreement between results of test performed on the same method items, by the same analyst, on the same instrument, under the same conditions in the same location and repeated over a short period of time. Repeatability represents “within-run precision”.

Intermediate precision refers to closeness of agreement between results of methods performed on the same method items in a single laboratory but over an extended period of time, taking account of normal variation in laboratory conditions such as different operators, different equipment and different days. Intermediate precision therefore represents “within-laboratory, between-run precision” and is therefore a useful measure for inclusion in ongoing validation.

Reproducibility refers to closeness of agreement between results of methods carried out on the same method items, taking into account the broadest range of variables encountered in real laboratory conditions, including different laboratories. Reproducibility therefore represents “inter-laboratory precision”.

In practical terms, internal laboratory validation will only be concerned with repeatability and intermediate precision and in many cases both can be investigated in a single series of well-designed experiments. Reduced precision indicates the presence of random error. The relationship between the components of analytical accuracy, types of error and the metrics used to describe them is illustrated in figure 5.

Any validation should also consider robustness, which, in the context of a quantitative method, could be considered as a measure of precision. However, robustness expresses how well a method maintains precision when faced by a specific designed “challenge”, in the form of precision does not represent random error. Typical variables in the laboratory include sample type, sample handling, sample quality, instrument make and model, reagent lots and environmental conditions (e.g., humidity, temperature). Appropriate variables should be considered and tested for each specific method. The principle of purposefully challenging methods is also applicable to both categorical and qualitative methods and

should be considered in the validation as well. Robustness can be considered as a useful prediction of expected intermediate precision (Berwouts, 2010).

As trueness and precision represent two different forms of error, they need to be treated in different ways. In practice, systematic error or bias can often be resolved by using a correction factor; constant bias requires an additive correction factor, whereas proportional bias requires a multiplicative correction factor.

For quantitative methods, particularly those requiring absolute quantification, it is most effective to estimate analytical accuracy on an ongoing basis by running a set of calibration standards (standard curve) with each batch or run. In this case, it is important that linearity be evaluated and that the lower and upper standards are respectively below and above the expected range of the results as precision cannot be assessed on extrapolated results. Where possible, calibration standards should be traceable to absolute numbers or to recognized international units.

Other factors that may need to be evaluated include the limit of detection defined as the lowest quantity of analyte that can be reliably detected above background noise levels and the limits of quantification that define the extremities at which the measurement response to changes in the analyte remains linear (Berwouts, 2010).

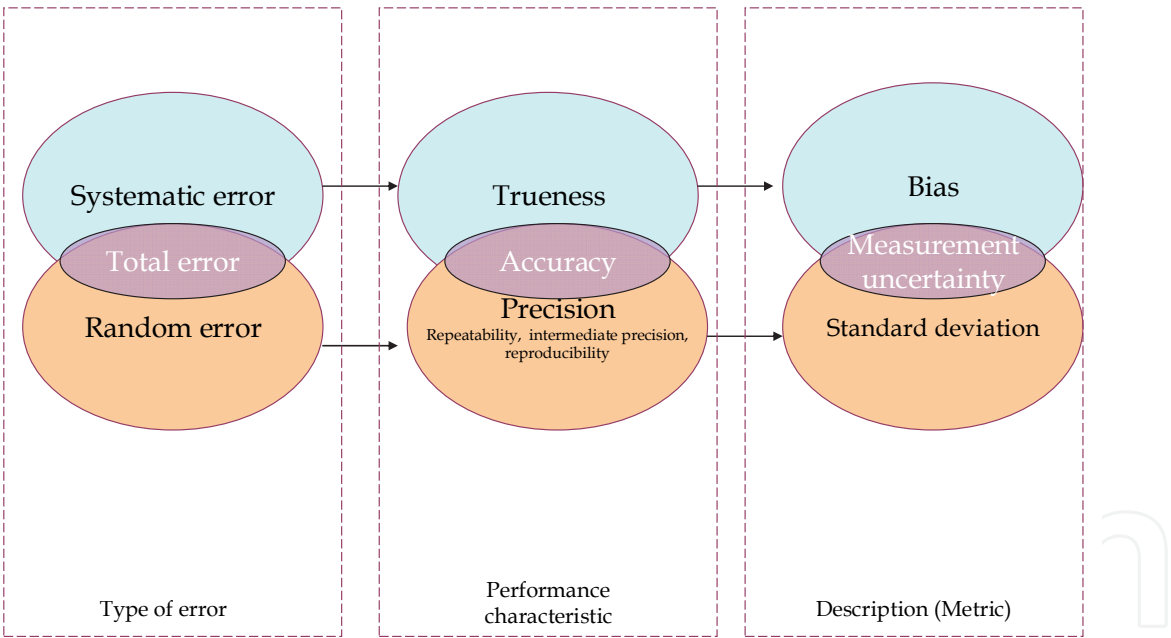


Fig. 5. Performance characteristics, error types and measurement metrics uses for qualitative methods (adapted from Menditto et al., 2007).

2.2.2 Qualitative methods

This is an extreme form of a categorical test/method, in which there are only two result categories, positive and negative. This binary categorization can be based either on a cut-off

to a quantitative result. The diagnostic accuracy of a qualitative method can be characterized by two components, both of which can be calculated: sensitivity (the proportion of positive results correctly identified by the method), and specificity (the proportion of negative results correctly identified by the method).

### 3. Parameters required for considering in the validation or revalidation of a method

There are several measurable parameters that should be taken into account during validation or verification. The estimation of *accuracy* is a key parameter. Accuracy consists of both precision and trueness for quantitative and semiquantitative test/method. *Precision* or “closeness of agreement between results of replicate measurements” includes the following:

*Repeatability*: within-run variation (same sample, same conditions).

*Intermediate precision*: between-run variation within a single laboratory (different samples, operators, equipments).

*Reproducibility*: between-run variation in different laboratories (different samples, operator).

*Robustness*: variation when confronted with relevant challenges (e.g., sample type, environmental conditions and so on).

*Trueness* is the “closeness of agreement with a reference value”. Appropriate reference materials are, therefore, essential and could include positive and negative/normal controls, certified reference materials, EQA materials, synthetic samples or material characterized by another technique.

The components of accuracy for quality tests/methods are *sensitivity* and *specificity*. *Sensitivity* is a measure of how well the test/method detects positive results, whereas *specificity* describes how well negatives are detected.

Thorough documentation during a validation process is essential, especially in the context of accreditation process pragmatic approaches, reconciling the formal requirements of accreditation standards while respecting the aim that “validation must be practical”, such as the design of IQC based on validation results, making full use of data that laboratories are already collecting, for example from IQC or EQA, for continuous validation. There are no detailed practical guides for validation of diagnostic methods in medical laboratories; moreover, the accreditation standards have no specific details about how to fulfil their requirements. The laboratory must decide on this, on the basis of their experience and performance requirements; it is duty of the laboratory to provide evidence that tests results provided are reliable, and that the performance claims are correct (Burnett, 2006; Hauck et al., 2008).

In the end, validation is never finished. The implementation of quality indicators for systematically monitoring and evaluating the laboratory’s contribution to patient care is good way to continuously validate diagnostic tests/methods, apart from IQC, EQA and other data (Eurachem, 2010; Hauck et al., 2008).

But when the method has already implemented several years and with performance and optimal quality specifications, we recommend periodic revalidation through the information provided by the treatment program reports or international interlaboratory comparison or testing fitness, programs or external quality control, in which the laboratory has been involved for years. The advantage of this validation methodology described above, is to estimate the imprecision, bias and uncertainty, without much effort and without having to make a lot of trials and experimental trials that would mean stopping the routine work in

the laboratory. You can validate the micro-range or specific concentration range, applicable as diagnostic daily reality for clinical laboratories.

### **3.1 Estimation of the accuracy using reference materials**

ISO 15189 accreditation for clinical laboratories require a verification of the accuracy of the measurement procedures. The study of the accuracy, by estimating the systematic error, should be in the validation of the measurement. To study the accuracy of a measurement procedure is necessary to compare average values obtained with a conventional true value. In the clinical laboratory can be used as true values considered, mainly 3 types: value assigned to a reference material, the consensus value obtained in a program of external quality assessment, the value obtained with a reference measurement procedure.

The accuracy is expressed numerically by systematic error, which is the difference between the average measurement results obtained and a conventional true value. The values assigned to some reference materials may be considered conventionally true values.

The reference materials used in the study must have a value assigned to the magnitude that is measured and the corresponding value of uncertainty. You should also know the traceability of the assigned value. It is preferable that the material has a matrix similar to human samples. The main types of materials: certified reference materials, prepared by metrology institutes or other organizations related to metrology and reference materials business (controls to the truth).

The reference material manufacturer must provide the traceability and uncertainty of assigned values; the latter expressed as standard uncertainty or expanded uncertainty. Along with the uncertainty value must also specify the coverage factor used.

The results of accuracy studies should be used to validate the accuracy of the measurement procedures, ensures the absence of relevant and introduce systematic errors in the calculation of uncertainty of measurement uncertainty components associated with any correction factors.

### **3.2 Estimation of the accuracy from participation in external quality assessment programs**

The external quality control includes different activities aimed at assessing the accuracy of the results through the intervention of an organization outside the laboratory. The most common form of external quality control is comparisons between laboratories or programs of external quality assessment.

These programs are organized by professional associations, government agencies or manufacturers of control materials that have a similar function. Participating laboratories measured once a magnitude of a control material of unknown value. Organization of the program collects the results of laboratory and a study of the data then forwarded to each participating laboratory, informing about the error of its outcome. The duration of the program, the number of measurements that are performed and the number of different materials are used, depending on the different programs. To study the accuracy is recommended that the program in which you participate fulfill the following conditions: high number of participants, the laboratory has a minimum of 12 results for participation and that you know the standard deviation characterizes the dispersion of results among participating laboratories.

### 3.3 Estimation of measurement uncertainty

The results provided by the clinical laboratory must be accurate (true and precise) to allow a correct clinical interpretation and to be comparable with earlier or later and between laboratories.

The error of measurement of clinical laboratory results is almost always unknown. Instead, it is possible to ascribe a measurement uncertainty and metrological traceability of each result. The uncertainty is a numerical expression of the degree of doubt of the result. Traceability relates the result with reference values established allowing reproducibility over time and between laboratories (Eurachem, 2010).

In the estimation of measurement uncertainty is assumed that any systematic error is eliminated, corrected or ignored, random effects are assessed on the outcome of an action and establishing a range within which lies the true value of the measured magnitude a certain level of confidence. The standard for laboratory accreditation ISO 15189 requires an estimate of the uncertainty of the results. The appropriate methodology for estimating the uncertainty described in the Guide to the Expression of Uncertainty in Measurement (GUM). The GUM was developed jointly by several international organizations for standardization and metrology for use in calibration and testing laboratories and measures applied to physical or chemical analysis. Currently, the GUM is difficult to apply to measures that are performed in clinical laboratories, although they maintained their principles. Moreover, the complexity and cost of obtaining an estimate of the uncertainty of measurement must be commensurate with the quality requirements applicable to the clinical use of the results.

Sources are contributing to the uncertainty of a result as follows: sample collection, sample preparation, calibrators or reference materials, input quantities (e.g., absorbance), computer equipment used, environmental conditions, sample stability and changes in workers.

The uncertainty associated with the collection and sample preparation is difficult to estimate and should be reduced through rigorous standardization of procedures. In this paper only consider the sources of uncertainty in the analytical phase, which begins when the sample interacts with the first technical step of the measurement (for example, placing the sample into an analyzer) and ends with obtaining a value numerical measurement result.

The main components of the uncertainty of the analytical phase correspond to the uncertainty of the measured, the stability of the sample in the measurement system calibration, the volume dispensed, the batch of reagents, instrumentation equipment, operators and environmental conditions. In the following paragraphs, are discussed in more detail the main components.

Measurement uncertainty is a parameter that is specifically associated with each outcome. In clinical laboratories, it is impossible to estimate particular measurement uncertainty for each measurand of each sample, so it makes a rough estimate of the uncertainty of measurement for a measurand defined and values of the same close to decision clinic. Measurement uncertainty does not apply to qualitative tests, in which the result is a numeric value.

#### 3.3.1 Definition of measurand

The measurand is defined by the following parameters:

- a. Analyte to be measured. For example, protein, sodium ion, cholesterol, ASO, hemoglobin, white blood cell counts, etc.
- b. System. For example, serum, urine, venous blood, pleural fluid, etc.



- c. Type size and unity. For example, substance concentration (mmol/L), mass concentration (g/L), catalyst concentration (nkat/L), etc.
- d. Measurement procedure.

The existence of different molecular forms of the analyte can introduce uncertainty in the results. This source of uncertainty can be reduced or eliminated by careful definition of the measurement, so they may react differently to some or other molecular forms.

Another source of uncertainty regarding the definition of the measurand are possible cross-reactions and interference that can occur with some samples and must be identified and documented to prevent, where possible, their influence.

In short, uncertainty caused by the uncertainty of the measurand can not be quantified, but may be reduced or eliminated by detailed specification of the measurand.

### 3.3.2 Imprecision

Most of the components of measurement uncertainty of the analytical phase are contained in the estimation of imprecision ( $CV_{id}$ ). It is usually obtained using control materials.

This assessment should be a sufficient number of data to collect the different sources of uncertainty apply, i.e., a minimum of six months of data and new estimation every year. In the period of data collection should include several calibrations to collect the uncertainty generated by the calibration process. Moreover it is necessary to use different batches of calibrator if you have the uncertainty of the assigned value.

The estimate of  $CV_{id}$  is made for a measurement value close to the values of clinical decision.

### 3.3.3 Value assigned to the calibrator

The clinical laboratory must know the uncertainty and metrological traceability of values assigned to calibration materials used. As usual it is commercial material the manufacturer must provide such data (Directive 98/79/EC). Along with the uncertainty value must also specify the coverage factor used. Typically, uncertainty is expressed as expanded uncertainty ( $U$ ) for a confidence level of 95% (coverage factor = 2).

The standard uncertainty ( $u$ ) is calculated by dividing  $U$  by the coverage factor.  $U$  on (%) of the value assigned to the gauge should not vary excessively batch to batch and should generally be lower than  $CV_{id}$ .

### 3.3.4 Systematic error (bias)

The estimation of measurement uncertainty is assumed that any significant systematic error of the measurement procedure has been deleted, corrected or ignored. The identification of a possible systematic error should be done during the validation of the measurement procedure.

When systematic error is corrected by a factor, the correction has an associated uncertainty ( $u_{cf}$ ) that should be considered in calculating the combined measurement uncertainty.

Systematic errors caused in the routine use of the measurement by the inevitable differences between different calibrations behave randomly in the long term, so this component of uncertainty is reflected in  $CV_{id}$ .

### 3.3.5 Uncertainty calculation

The uncertainty is calculated by combining various sources. For this reason, clinical laboratories should identify each measurand, specifying the measurement procedure, and



calculate for each of them calculate the combined uncertainty from the data of internal quality control and other data, using the following equation:

$$u_c = \sqrt{CV_{id}^2 + u_{cal}^2 + u_{cf}^2}$$

Where:

$u_c$ : relative combined standard uncertainty (%);  $CV_{id}$ : imprecision (coefficient of variation) interday;  $u_{cal}$ : relative standard uncertainty (%) of the value assigned to the calibrator;  $u_{cf}$ : relative standard uncertainty (%) of the factor used to correct a systematic error. It is recommended to express the combined uncertainty for a confidence level of 95% (expanded uncertainty,  $U_c$ ). To do this, multiply the value of  $u_c$  for  $k = 2$ .

$$U_c = 2 \times \sqrt{CV_{id}^2 + u_{cal}^2 + u_{fc}^2}$$

The relative expanded uncertainty should be expressed to two significant figures, for example: 4.2%, 16%.

### 3.3.6 Interpretation

The estimation of measurement uncertainty provides a quantitative indication of the level of doubt that the laboratory has in each result and is therefore a key element in the system of analytical quality in clinical laboratories.

The relative expanded uncertainty of a measurand should be less than one third of the Maximum Permitted Error (MPE). If it was superior, should be studied in greater detail the different sources of uncertainty, identify the most significant and perform the appropriate actions to reduce them.

### 3.3.7 Applications

The uncertainty of measurement should be used primarily for:

- Selection of measurement procedures that fulfill the specifications of accuracy.
- Strict interpretation of the significance of a change between two consecutive values of magnitude biochemistry.
- Strict interpretation of the significance of a result compared with a value of clinical decision.

### 3.3.8 Limitations

The value of the measurement uncertainty varies with the concentration of the measurand and may be substantially different for very low or very high analyte. For this reason it is recommended that the estimate for a concentration closes to clinical decision values.

## 3.4 Estimation of precision

Precision is one of the most important metrological characteristics to be considered for selection and implementation of a measurement procedure in the clinical laboratory. In addition, the quantitative understanding of this feature is essential for establishing tolerance intervals of internal control materials for the objective interpretation of the significance of a change between two consecutive values of magnitude biochemistry, and the calculation of uncertainty.

The accuracy can be studied under conditions of repeatability, reproducibility and intermediate. The study conditions that are more interested in the clinical laboratory are the kind of repeatability and intermediate terms, which vary from day to day.

Before starting the study a period of familiarization with the measurement procedure and the operator's experience are recommended.

Also for this study is recommended that samples for this study were commercial materials or control samples. We recommend using at least two samples with different concentrations of the magnitude under study, with a value within the physiological range or close to a discriminate value, and one with a pathological value. When it sees fit, they can not be tested with values close to the limits of the measuring range of the procedure. Samples should be stable during the duration of the study. If using commercial control materials, whenever possible, they should be interchangeable with samples of human origin.

The run imprecision were obtained under the same conditions of repeatability, ie the same samples, the same operator, the same components of the measurement system for a short time and without calibrations between measurements. A minimum of 30 measurements are required for fulfilling statistical criteria.

If the results of the run imprecision are not consistent with previous results, either supplied by the manufacturer or obtained in the literature, the study should be stopped to find and correct the cause of the discrepancy.

Imprecision is obtained under certain conditions. Each laboratory should perform the estimation with standard calibration frequency (daily weekly, etc), and changes of operator, calibrator lot, reagent lot, etc, which are common in everyday work. Following the statistical criteria are recommended to estimate a minimum of 30 days.

Before calculating the mean, standard deviation and coefficient of variation of the results must be detected the presence of possible outliers. An abnormal result will be removed provided that it is related to a documented error or has demonstrated statistically that is an outlier. After the removal of outliers, if any, imprecision is calculated by the coefficient of variation.

#### **4. Documentation: procedures and instructions needed for the validation of a method in the clinical laboratory**

International Standard ISO 15189:2007 clearly identifies the documentation requirements necessary to determine compliance with the requirements referred to for quality and competence of clinical laboratories.

Standard clinical laboratory means (paragraph 3.8) that "laboratory devoted to biological, microbiological, immunological, chemical, immuno-hematological, biophysical, cytological, pathological or other material derived from the human body in order to provide diagnostic information, prevention and treatment of diseases or the assessment of human health and can provide a consultant advisory service covering all aspects of laboratory analysis, including interpretation of the findings and recommendations on any proper analysis additional".

The implications of documentary that suggests the validation of a method, it follows that it must develop a set of documents or records.

Registration means that documentary evidence of a fact that has occurred and is understood by documentary evidence to document that describes how the activities should be conducted.

According to these definitions it is able to state that the laboratory should have a defined overall validation procedure (document) that describes: What activities will be performed; responsibility to perform; records to retain; how to be performed.

To confirm the verification of compliance, apply the method to real matrices, records to keep are: 1. Requirements applied to the method (Must be defined prior to conducting the tests, indicating preserved based on what have been defined); 2.Records of previous tests. (Straight calibration standards used, results obtained from different computers, etc.); 3. Written procedure (approved by qualified personnel); 4. Results of tests for checking compliance with requirements (The laboratory must clearly indicate the results of the parameters and the comparison with the specified requirements); 5. Statement by the head of the validation of the procedure is suitable for their intended use based on the evidence (All these records should include dates, personnel and equipment used in ways that can be reconstructed).

It must have an overall validation procedure describing the activities undertaken; those responsible for conducting, records to keep (the method established requirements, records of tests: calibration lines, patterns, etc.).

Lab logo	Registration	"Name of the Laboratory"
	Design/planning of the method validation	Date:
		CODE:
		Review:
Page X of Y		
Method:		
Responsible for validation:		
Used:		
References:		
Procedure/codification:		
Objectives:		
Parameter	Value	Observations

Fig. 6. Template record: Design / planning of the method validation.

It must be developed and used a generic template so that it is not necessary to have to develop a validation process for each method, but simply change the data in the template. Thus, for any method in the laboratory which will continue to want to validate one of the two ways described: the classical or from the results of inter-comparison programs. It is used for the models listed in the Annexes to this case: report validation, design / planning

of the method validation, quality control plan method. They detail steps for each procedure to be followed in the validation.

Here are a few examples of formats and / or templates of records that are considered necessary for the validation of a method:

- Template record: Design / planning of the method validation (figure 6).
- Template record: Report of validation (figure 7).
- Registration: Plan quality control method (figure 8).
- Title page of a validation procedure in clinical laboratory methods (figure 9).
- Technical Registration. Spreadsheets (Excel) (figure 10).

LAB LOGO	VALIDATION REPORT		"Name of the laboratory"
			Date:
			Code:
			Review
			Page X of Y
Date of procedure of validation	Register	Observations	Personnel
Additional data			
Parameter	Result /value	Observations	
Declaration: <input type="checkbox"/> Validated method <input type="checkbox"/> No validated method <input type="checkbox"/> Validated method with restrictions			
Validation criteria:			
Date of validation of the method:		Signature: (Responsible of validation)	

Fig. 7. Template record: Report of validation.



LAB LOGO	MANUAL OF PROCEDURES	"Name of the laboratory"
	PROCEDURE FOR VALIDATION METHODS	Date:
		Code:
		Review
		Page X of Y

CONTENTS

1. OBJECTIVE AND SCOPE

2. DEFINITIONS

3. REFERENCE DOCUMENTS

4. PROCEDURE

4.1. Accuracy

4.2.Precision

4.3.Trueness

4.4. Repeateability

4.5.Reproducibility

4.6. Uncertainty

4.7. Another validation parameters

5. ANNEXES

Fig. 10. Contents in a validation procedure in clinical laboratory.

5. Conclusion

It is important to have documented procedures for the validation of different diagnostic methods available within a clinical laboratory. There is a need to develop practical guidelines for method validation procedures in clinical laboratories through the various tools available to the laboratory. There is no single way to validate a diagnostic and clinical laboratory validate and verify the validation of their methods over time to meet the requirements of the existing accreditation standards and to demonstrate the laboratory's technical competence to offer quality results.

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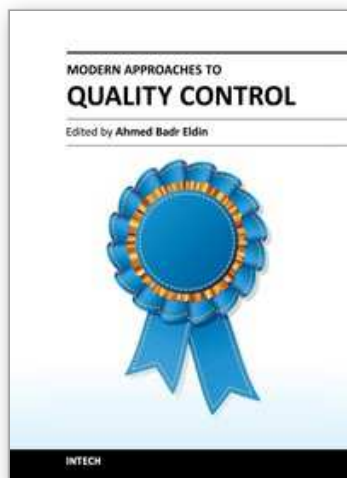
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