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Verification of examination procedures in clinical laboratory for imprecision, trueness and diagnostic accuracy according to ISO 15189:2012: a pragmatic approach

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Abstract

Background: The International Standard ISO 15189 is recognized as a valuable guide in ensuring high quality clinical laboratory services and promoting the harmonization of accreditation programmes in laboratory medicine. Examination procedures must be verified in order to guarantee that their performance characteristics are congruent with the intended scope of the test. The aim of the present study was to propose a practice model for implementing procedures employed for the verification of validated examination procedures already used for at least 2 years in our laboratory, in agreement with the ISO 15189 requirement at the Section 5.5.1.2.

Methods: In order to identify the operative procedure to be used, approved documents were identified, together with the definition of performance characteristics to be evaluated for the different methods; the examination procedures used in laboratory were analyzed and checked for performance specifications reported by manufacturers. Then, operative flow charts were identified to compare the laboratory performance characteristics with those declared by manufacturers.

Results: The choice of performance characteristics for verification was based on approved documents used as guidance, and the specific purpose tests undertaken, a consideration being made of: imprecision and trueness

for quantitative methods; diagnostic accuracy for qualitative methods; imprecision together with diagnostic accuracy for semi-quantitative methods.

Conclusions: The described approach, balancing technological possibilities, risks and costs and assuring the compliance of the fundamental component of result accuracy, appears promising as an easily applicable and flexible procedure helping laboratories to comply with the ISO 15189 requirements.

Keywords: accreditation; examination procedures; ISO 15189; verification.

Introduction

The International Standard ISO 15189 is recognized as a valuable guide in ensuring high quality clinical laboratory services, and in promoting the harmonization of accreditation programs in laboratory medicine. The development and implementation of a specific standard for clinical laboratories adds value to the entire system of quality management of laboratories, and also enhances patient safety [1, 2].

The main objective of ISO 15189, patient care, is attained by laboratories' verification of their management of organization/responsibilities and technical quality, and their pursuit of continual improvement [3]. The implementation of a quality system complying with the ISO 15189 calls for the verification or validation of examination procedures (Sections 5.5.1.2 and 5.5.1.3) as the means to guaranteeing that their characteristics meet the specifications obtained during the validation [1].

Most of the examination procedures used in the medical laboratory originate from in vitro diagnostic (IVD) methods from the manufacturers, without being modified; only a few are developed "in-house" or a modified versions of the methods specified by manufacturers.

The third Medical Device Directive, the IVD Medical Device Directive 98/79/EC [4] regulates, IVD medical devices in Europe, and has been mandatory since December 2003. Manufacturers are responsible for validating

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the performance of instruments and reagent kits, the aim being “*to make systems of measurement having metrological characteristics better than those currently in the market*”; medical laboratories, on the other hand, must specify requirements (performance specifications) during the procurement process. According to the Directive, the devices must take “*into account the generally acknowledged state of the art,*” and in order “*to fulfil the intended purpose of the examination procedure*” must achieve the performances, where appropriate, in terms of analytical sensitivity, diagnostic sensitivity, analytical specificity, diagnostic specificity, accuracy, repeatability, reproducibility, including control of known relevant interference, and limits of detection.

In addition, according to the ISO 15189, the Directive requires that the IVD device in the hands of its end users must achieve the performance stated by the manufacturer. In particular, the ISO 15189 states that “*The laboratory shall obtain information from the manufacturer/method developer for confirming the performance characteristics of the procedure. The independent verification by the laboratory shall confirm, through obtaining objective evidence (in the form of performance characteristics) that the performance claims for the examination procedure have been met. The performance claims for the examination procedure confirmed during the verification process shall be those relevant to the intended use of the examination results*” [1].

A pragmatic approach based on the awareness that laboratory results must be reliable is needed in order to promote the use of ISO 15189 in clinical laboratories and to define reliable, user-friendly operating procedures.

Aim

The aim of the present study was to propose a practical model for implementing procedures designed to verify examination procedures in agreement with the ISO 15189 requirement at the Section 5.5.1.2, the verification being limited to those already in use for at least 2 years, while taking into account the heterogeneity of the methods and harmonizing this procedure.

The model described in the present study has been used in the Department of Laboratory Medicine of the University Hospital of Padova, with a turnover of about 8,700,000 examinations/year, of which 86% are under a routine and 14% an emergency regime, and about 50% for both outpatients and inpatients. The Department obtained accreditation in accordance with the Standards of the Clinical Pathology Accreditation of the United Kingdom (CPA-UK) in 1995 [5], and certification in accordance with

the International Standard ISO 9001 in 1997 [6]. In 2016 the laboratory was accredited for the majority of the tests conducted in various laboratory medicine specialties in compliance with the ISO 15189:2012 [1].

Materials and methods

In order to identify the performance characteristics to be assessed and the operative procedure to be used to verify the examination procedures in the laboratory, the following steps were taken:

1. Identification of approved documents to define the operative flow;
2. Definition of performance characteristics to be evaluated for the different methods and analytes;
3. Analysis of examination procedures used in laboratory, splitting into categories, and checking for performance specifications reported by manufacturers;
4. Identification of operative flow charts to evaluate the performance characteristics in relation to each typology of method, and to compare them with those declared by manufacturers.

Results

Identification of approved documents to define the operative flow

A literature search was made in order to identify the approved documents to use as a guide. Numerous documents were selected [7–30] and, after an analysis of the contents, the following were identified for reference to define the procedure of analytical methods verification:

- Eurachem “*The fitness for purpose of analytical methods – A laboratory guide to method validation and related topics*” [7];
- ISO/DTS 21748 “*Guide to the use of repeatability, reproducibility, and trueness estimates in measurement uncertainty estimation*” [8];
- Accredia DT-07-DL/DS “*Guide to perform tests with qualitative results*” [9];
- CLSI EP15 “*User verification of precision and estimation of bias*” [19];
- CLSI C24 “*Statistical quality control for quantitative measurement procedures*” [21].

Definition of performance characteristics to be evaluated for each group of methods

The choice of parameters to verify for all methods used in our laboratory is based on the evidence that the accreditation process is developed in a laboratory in which the

quality system has already been: implemented according to the International Standard ISO 9001:2008; accredited in compliance with the Clinical Pathology Accreditation Standards CPA-UK [5, 6]. Therefore the criteria identified and procedures carried out pertain to a laboratory in which the process is under control, users' satisfaction is evaluated and an improvement process is underway. Consequently, if the examination procedures used provide results that satisfy clinical needs and, consequently, the purpose of the test, the verification can be limited to the parameters highlighting the accuracy of results.

For *quantitative methods* (that determine the amount of a specific substance present in a sample under analysis), the evaluation of imprecision (in terms of coefficient of variation, CV%) and trueness (in terms of Bias%) of examination procedures are identified as minimal performance characteristics to be measured and compared with values declared by manufacturers.

For *qualitative methods* (characterized by two possible answers: positive/negative, presence/absence, reactive/non-reactive, yes/no, etc.), and diagnostic accuracy (in terms of diagnostic sensitivity and specificity) were considered performance characteristics for verification, as reported in documents issued by Accredia [9], while following the CLSI EP12 guideline [18].

For *semi-quantitative methods* (that combine features of quantitative and qualitative methods and generate raw data quantities), diagnostic accuracy and imprecision were verified.

Analysis of examination procedures used in laboratory and grouping into categories and checking performance specifications reported by manufacturers

The examination procedures used in our laboratory (645 tests) were analyzed and grouped into the following categories: quantitative (67%), semi-qualitative (6%) and qualitative (14%). Moreover, 13% of examination procedures that were followed gave rise to an interpretative comment.

For *quantitative methods*, 89% of tests had more than 20 internal quality control (IQC) values in a year, and the manufacturers reported the imprecision value for each (CV%). Ten per cent of tests had less than 20 IQC values, or IQC was not available. In the remaining 1% of cases, the tests had more than 20 IQC values, but the manufacturer did not report the CV%. Seventy-seven per cent of the quantitative tests were monitored by external quality assurance system (EQAS), but the manufacturer

did not declare the Bias percentage (Bias%) for trueness. The Bias% was reported for only one quantitative test (0.2%), for which EQAS was available. In 22.8% of cases, tests were without EQAS and no trueness value was declared.

For *qualitative methods*, EQAS was not available for 30% of the tests and the manufacturer provided diagnostic accuracy values, while for 55% of tests, EQAS was available but the manufacturer did not declare the diagnostic accuracy. The remaining 15% accounted for tests without both EQAS and a claimed diagnostic accuracy.

For imprecision verification of *semi-quantitative methods*, 27% of tests had more than 20 IQC values, for which the manufacturer provided the imprecision value. Five per cent of tests had less than 20 IQC values or IQC were not available. The remaining 68% accounted for tests with more than 20 IQC values, for which the manufacturer did not state the imprecision value. To verify diagnostic accuracy, for only 16% of the tests EQAS were available, and the manufacturer declared diagnostic accuracy in these cases. Fifty-nine per cent of tests had EQAS but the manufacturer did not declare diagnostic accuracy while for 16% of these tests, EQAS were not available, although the manufacturer did declare the diagnostic accuracy. The remaining 8% were tests without EQAS and claimed diagnostic accuracy.

For verification of *tests with interpretative comments*, the many tests (58%) have EQAS, while the remaining tests are without EQAS.

Identification of operative flow chart to evaluate the performance characteristics and compare them with those declared by manufacturers

A flow chart was defined for each type of examination procedure in order to evaluate the performance characteristics chosen. Regarding **imprecision verification** of *quantitative and semi-quantitative methods*, the flow charts are reported for the methods with more than 20 IQC values, and for which the manufacturer specified (Figure 1) or did not specify (Figure 2) imprecision. Figure 3 shows the flow chart for methods with <20 values of IQC or without IQC. The IQC values were chosen on the basis of concentration levels that were quite similar to those specified by the manufacturer, and, if possible, close to the decisional level of the specific analyte.

When the laboratory's imprecision ($CV\%_{LAB}$) was greater than that specified by the manufacturer ($CV\%_{manuf}$), its acceptability was evaluated by calculating the ratio $CV\%_{LAB}^2 / CV\%_{manuf}^2$ and by comparing this value with those

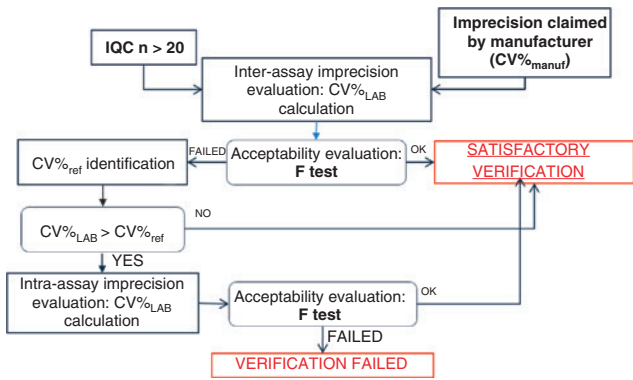


Figure 1: Operative flow for imprecision verification (quantitative and semi-quantitative examination procedures) when more than $n = 20$ IQC are available and when the manufacturer claims the imprecision.

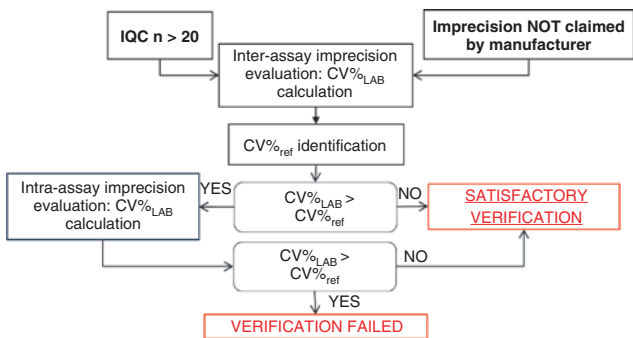


Figure 2: Operative flow for imprecision verification (quantitative and semi-quantitative examination procedures) when more than $n = 20$ IQC are available and when the manufacturer does not claim the imprecision.

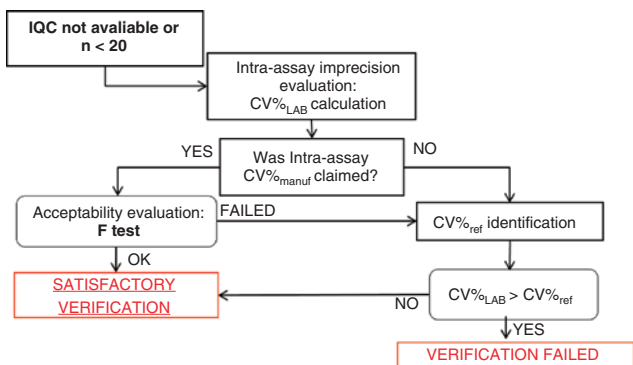


Figure 3: Operative flow for imprecision verification (quantitative and semi-quantitative examination procedures) when less than $n = 20$ IQC are available or IQC are not available.

retrieved from an F distribution, with 95% as significance level and the appropriate degrees of freedom for numerator and denominator ($F_{1-\alpha, n1-1, n2-1}$) [19]. The F distribution

was used to test the null hypothesis of equality of CVs, and the laboratory result was considered inappropriate if the calculated ratio $CV\%_{LAB}^2 / CV\%_{manuf}^2$ was higher than the $F_{1-\alpha, n1-1, n2-1}$ value.

When it was impossible to compare $CV\%_{LAB}$ with $CV\%_{manuf}$ or the F test showed significant differences, the $CV\%_{LAB}$ was compared with a “reference” CV% ($CV\%_{ref}$), identified following the hierarchical structure established in the 1999 Stockholm Consensus Conference [31, 32], on the basis of: (a) clinical recommendations; (b) biological variation; (c) state-of-the-art. An alternative approach is to define the analytical limit for meeting medical utility, defined in 1985 by Skendzel et al. as “medical CV” [33], which was recently reviewed and re-proposed by Klee [34]. The appropriateness of the identified $CV\%_{ref}$ should be evaluated in relation to the intended scope of the examination procedures.

The CV% value calculated on laboratory IQC data is a less stringent imprecision than that declared by the manufacturer because it involves different reagent lots, several operators, and several days to finalize. Therefore, a reliable comparison between laboratory imprecision calculated on the basis of IQC data and imprecision declared by manufacturers guarantees satisfactory performance.

When the comparison between the $CV\%_{LAB}$ from IQC data and the inter-assay $CV\%_{manuf}$ is not satisfactory and when $CV\%_{ref}$ is not identified or is higher than $CV\%_{LAB}$, the intra-assay $CV\%_{LAB}$ can be calculated: IQC or patient samples are analyzed at least ten times in one analytical run, or by combining the intra-assay CVs% as suggested by CLSI in the EP15 [19, 35]. The intra $CV\%_{LAB}$ is compared directly with the intra $CV\%_{manuf}$, or the F test is used, as previously detailed.

The operative condition of intra-assay imprecision tests should be similar to that of the manufacturer: the results should reflect the best possible performance for imprecision for the routine laboratory.

For the verification of **trueness** of *quantitative methods*, three operative flow charts are proposed, considering the availability or unavailability of the EQAS for calculating the Bias%, and the availability of the Bias% specified by the manufacturer ($Bias\%_{manuf}$), as illustrated in Figures 4–6.

The laboratory’s bias ($Bias\%_{LAB}$) is calculated on the basis of EQAS results from at least eight samples in relation to the target value of the specific diagnostic system used in laboratory for those diagnostic systems where the target value obtained with the reference procedure (reference value) is not available or for those diagnostic systems that highlight standardization problems (high $Bias\%$ between consensus value and reference value) or

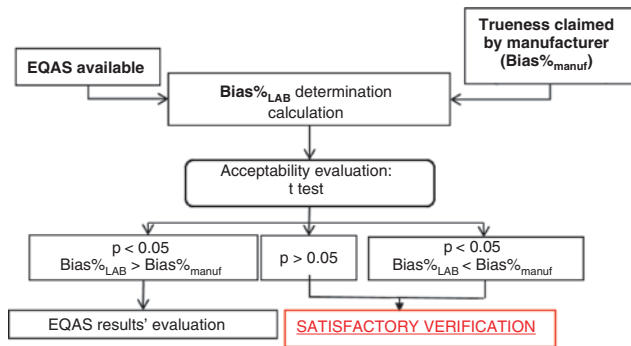


Figure 4: Operative flow for trueness verification (quantitative examination procedures) when EQAS are available and when the manufacturer claims the trueness.

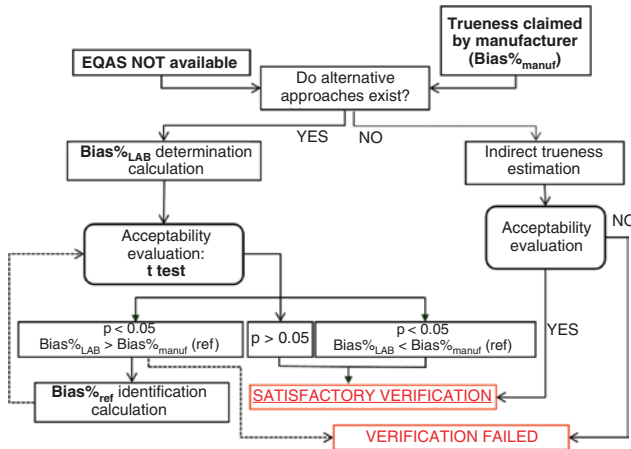


Figure 5: Operative flow for trueness verification (quantitative examination procedures) when EQAS are not available and when the manufacturer claims the trueness.

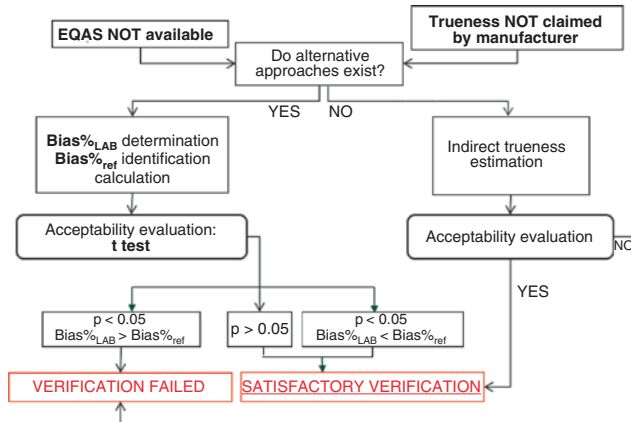


Figure 6: Operative flow for trueness verification (quantitative examination procedures) when EQAS are not available and when the manufacturer does not claim the trueness.

when control material is not commutable for the specific diagnostic system.

When the comparison between the laboratory’s ($Bias\%_{LAB}$) and the manufacturer’s ($Bias\%_{manuf}$) Testo Bias is not satisfactory or is not possible, a “reference” Bias% ($Bias\%_{ref}$) can be identified as described above for imprecision verification, using the criteria identified in the 1999 Stockholm Consensus Conference [31, 32].

Thereafter, for each $Bias\%_{LAB}$ computed, a calculation should be made of the difference between $Bias\%_{LAB}$ and the comparison bias ($Bias\%_{manuf}$ or $Bias\%_{ref}$). The mean and standard deviation resulting from these differences were estimated. The acceptability of the verification is performed according to ISO 21748 by using a two tail t-test at 0.05 significance level, considering a mean equal to zero as null hypothesis [8]. Three different scenarios are possible:

- 1) $p > 0.05$ and $Bias\%_{LAB} < Bias\%_{manuf}$: data are comparable and the verification is successful;
- 2) $p < 0.05$ and $Bias\%_{LAB} < Bias\%_{manuf}$: laboratory’s results are better than manufacturer’s data and the verification is successful;
- 3) $p < 0.05$ and $Bias\%_{LAB} > Bias\%_{manuf}$: laboratory’s results are worse than manufacturer’s data and the verification is unsuccessful – in this case, the EQAS results are evaluated, considering the acceptability criteria of the EQA institution, this approach being used also when the manufacturer does not claim the trueness, but EQAS results are available.

When the EQAS are not available, $Bias\%_{LAB}$ can be calculated by means of alternative approaches, as suggested by CLSI [21]: comparison with (a) certified reference material with demonstrated commutability versus the test samples, (b) reference method or (c) another routine laboratory method. However, it is often impractical to calculate $Bias\%_{LAB}$ with these methodologies: another way is to indirectly estimate the trueness as proposed by Eurachem and CLSI [7, 19], considering the specific sample and methodological characteristics in order to assure reliable results. Dilution and recovery tests can be carried out if a pure measurand is available and can be spiked into a suitable patient-like matrix, following, when it is possible, the instruction reported by manufacturers. The results obtained are evaluated in relation to the intended use but the extent of the recovery should be consistent, precise and reproducible [12].

For the verification of the **diagnostic specificity (Sp) and sensitivity (Se)** of *qualitative and semi-quantitative methods*, two flow charts were developed for tests with diagnostic accuracy stated or not stated by the manufacturer (Figures 7 and 8, respectively). When the

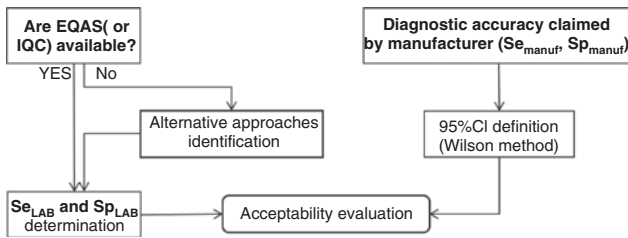


Figure 7: Operative flow for diagnostic accuracy verification (qualitative and semi-quantitative examination procedures) when the manufacturer claims the diagnostic accuracy. Se, diagnostic sensitivity. Sp, diagnostic specificity.

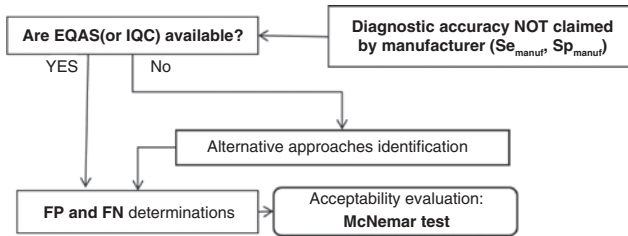


Figure 8: Operative flow for diagnostic accuracy verification (qualitative and semi-quantitative examination procedures) when the manufacturer does not claim the diagnostic accuracy. Se, diagnostic sensitivity. Sp, diagnostic specificity.

manufacturer reports diagnostic accuracy performances (Se_{manuf} , Sp_{manuf}) together with the total number of specimens evaluated, the Se_{manuf} and Sp_{manuf} 95% confidence intervals (95% CI) are calculated using the Wilson method [36].

When EQAS are available, laboratory diagnostic accuracy (Se_{LAB} , Sp_{LAB}) can be assessed in relation to the designated response of the scheme, considering at least ten samples, even if a larger sample size is advisable to increase the diagnostic accuracy estimation. Alternatively the Se_{LAB} and Sp_{LAB} can be calculated using IQC results, but only if at least 10 positive and at least ten negative IQC values are available: this allows a comparison between Se_{manuf} and Sp_{manuf} on the basis of the respective 95% CI. The verification is successful when:

- Se_{LAB} and Sp_{LAB} are both included in the 95% CI of Se_{manuf} and Sp_{manuf} ;
- Se_{LAB} and Sp_{LAB} are both greater than the upper confidence bounds of Se_{manuf} and Sp_{manuf} ;
- Se_{LAB} or Sp_{LAB} is included in the 95% CI of Se_{manuf} and Sp_{manuf} and the other is greater than the upper confidence bound.

If only one of Se_{LAB} and Sp_{LAB} falls within the Se_{manuf} and Sp_{manuf} 95% CI, while the other is outside the lower range, an analysis must be made of the specific intended use of

the test (screening, diagnosis or monitoring). For screening tests, the Se_{LAB} should be included to the 95% CI of Se_{manuf} ; for diagnostic tests, the Sp_{LAB} should be included in the 95% CI of Sp_{manuf} ; for monitoring tests, acceptability is assessed in relation to the specific test.

Diagnostic accuracy not reported by the manufacturer can be verified by applying McNemar's test as recommended by CLSI [18], the EQAS being considered the reference method and a calculation being made of the false positive and false negative results.

When EQAS are not available and the manufacturer does not claim diagnostic accuracy, it is also possible to calculate the Se_{LAB} and Sp_{LAB} using alternative approaches, but only if a cut-off is declared for the test: certified reference material or a quantitative method can be used to obtain the number of false positive and false negative determinations in order to apply McNemar's test as described above. Otherwise a selection can be made of samples with a confirmed diagnosis (true positive) and samples without disease (true negative), and the test diagnostic accuracy verified.

For examination procedures that provide an interpretative comment, the verification of congruity of the comment in relations to the patient health conditions should be checked. When this is not possible, a satisfactory evaluation in the EQAS can represent a satisfactory result in the verification process; when EQAS is unavailable, the congruity of the comments can be verified by evaluating the consensus among the answers provided by different operators using selected patient samples [37].

Discussion

The current limited or unavailable evidence of specific guidelines that should help clinical laboratories to comply with the ISO 15189 requirements obliges laboratories to define internal procedures by reference to approved recommendations that are often complex, costly and inconsistent with clinical needs.

From the perspective of a clinical laboratory, on verifying examination procedures, it must be taken into account that the implementation of the accreditation process in compliance with the ISO 15189 takes place in a context where an audited quality system is already in place and results provided to users do not give rise to serious complaints.

On the other hand, the decision to verify at least imprecision and trueness for quantitative methods, and the diagnostic accuracy and imprecision for semi-quantitative methods, appears appropriate for assuring the

reliability of the diagnostic system according to validation specifications. The verification of these parameters [30], arises from the awareness that they are important in highlighting any casual and systematic errors, and supporting standardization of test methods, thus providing the information needed to estimate the uncertainty of results, allowing the comparison of performance of different methods [38]. The verification of diagnostic sensitivity and specificity for qualitative and, in part, for semi-quantitative methods, appears appropriate assuring the quality of results, with respect to their intended use [9]. In addition, assessing imprecision/trueness or sensitivity/specificity could be a good compromise in guaranteeing the reliability of results of the used methods, as it contains costs and is less time-consuming for laboratories that intend to apply for ISO 15189 accreditation.

In order to investigate the above, we initially evaluated and discussed several guidelines, identifying several documents, used to formulate the verification process of examination procedures. On selecting documents, we also considered the possibility of identifying reliable statistical methods for assessing differences between the laboratory's and the claimed performances.

The heterogeneity of tests used in our laboratory was highlighted while during operative flow preparation, clinical chemistry accounting for 38%, clinical pathology 32%, immunology 29%, and clinical molecular biology 1%. Quantitative, qualitative, semi-quantitative tests and methods with interpretative comments were evaluated; then tests between 1 day and 1 month of report time were considered. In developing the model, whether or not the manufacturer had declared imprecision, trueness and diagnostic accuracy were considered. As an example, for qualitative methods, the diagnostic accuracy with all the parameters necessary for the calculation (number of true negatives, true positives with total number of patients, total number of "healthy" subjects) was stated in 32% of tests. Furthermore, for only one quantitative test, a trueness was declared. Appropriate flow charts have therefore been proposed for all conditions described.

This operating process for verifying the examination procedures has several (potential) limitations. In particular, its complexity is increased by the identification of different flow charts in relation to the method typologies and to the availability of performance specifications by the manufacturer.

Our approach has been applied for the methods already in use in laboratory. Whenever a new method is introduced, a different, more rigorous approach will be applied. Moreover, since the statistical methods used call for expert skills, and can be time consuming; it would therefore be advisable

to generate spreadsheets designed to meet all the various requirements. Regarding the non-homogeneity of EQAS reports, it can be difficult to extrapolate the data necessary for calculating $\text{Bias}_{\text{LAB}}\%$, and, in some cases, not all the data were available: an effort should therefore be made to improve the EQAS harmonization process [39].

Conclusions

The International Standard ISO 15189, the recognized guideline for medical laboratories accreditation, is not extensively implemented because it involves a demonstration of the technical suitability of tests, increasing the workload of staff and entailing substantial costs. Operating procedures based on a pragmatic approach, where appropriate, are required in order to encourage laboratories to implement the accreditation process for all tests provided. However, a more stringent approach is required for newly introduced tests, and, when a laboratory procures a diagnostic system, it must ensure that the manufacturer provides clear, complete quality specifications.

The approach proposed in the present study aims to help clinical laboratories use an easily applicable and flexible system in which the method verification demonstrates that the examination procedure is fit for its intended purpose, balancing technological possibilities, risks and costs. This model should be promptly simplified, with an update of the methods datasheet by manufacturers.

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