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GENERAL PAPER

Analytical validation of tests in laboratories of anatomic pathology: a Belgian population-based study

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Abstract
Laboratories of anatomic pathology have to validate each analytical test before it is implemented in daily routine. However, evidence-based guidelines regarding validation of analytical tests are limited. A 2016 survey on test validation was performed in all 77 licensed Belgian anatomic pathology laboratories in order to evaluate the implementation and to highlight the current issues of test validation in Belgian anatomic pathology laboratories. Therefore, standard operating procedures for test validation and validation reports were evaluated for the presence of predefined items. Separate validation procedures for CE/IVD-labeled, laboratory-modified and laboratory-developed tests and a description of how each performance characteristic was validated were lacking in 51 (66 %) laboratories. Moreover, only 9 (12 %) laboratories reported to have written procedures for when and how analytical tests need to be revalidated. Better results were observed regarding the description of the performance characteristics, the co-workers involved, recording and archiving of results and raw data, the content of the validation report, ongoing validation, release and implementation into daily routine. Contrary to the evaluation results of the procedures for test validation, better results for the content of the validation reports were obtained. A lack of clear and predefined objective acceptance criteria for each determined performance characteristic was the most common shortcoming observed in validation reports in Belgian anatomic pathology laboratories. In conclusion, there appears to be a need for further development of guidelines for validation and revalidation of analytical tests performed in anatomic pathology laboratories.

Keywords Laboratory of anatomic pathology · Test validation · Standard operating procedure · Validation report

Introduction

Medical laboratory services are essential for patient care and therefore should meet the needs of all patients and clinical personnel responsible for human health care. In order to monitor and guarantee the quality of the performance of Belgian laboratories of anatomic pathology, conditions for good laboratory performances are imposed by the Royal Decree (RD) of December 5, 2011, regarding the license of anatomic pathology laboratories which took effect on March 1, 2013. After a transition period of 1 year, all Belgian anatomic pathology laboratories are licensed since March 1, 2014. Within the framework of these licensing, laboratories are obliged to elaborate a quality management system within 5 years. Therefore, the National Commission of Anatomic Pathology provided to the laboratories a practical guideline with requirements for competence and quality based on the International Standard ISO 15189:2012 and that are particular to laboratories of anatomic pathology. It provides a framework for the design and the improvement of a quality management system in the laboratories of anatomic pathology.

Ensuring the technical adequacy of the obtained results is one of the main legal requirements, which is also elucidated in the practical guideline. In order to deliver technically valid results in a consistent way, each test has to be validated before being used for analyzing patient specimens. The purpose of test validation is to demonstrate that the new analytical test meets the predefined criteria based on the manufacturer's specifications, literature data, etc. within the laboratory. However, the process of implementing a test for diagnostic use may be complex. The complexity and the extent of a validation procedure will depend on the fact whether the test is CE/IVD labeled or not. When initial testing shows that the manufacturer's specifications of a CE/

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IVD-labeled test cannot be met, the laboratory may decide to modify one or more prescribed specifications in order to optimize the test. In contrast with a CE/IVD-labeled test strictly applied according to the manufacturer's specifications and for which a verification of the accuracy, characterized by the measurement of the trueness and the precision (including measurement of repeatability, intermediate precision and reproducibility), is sufficient, a more extensive validation procedure has to be applied in the case of a laboratory-modified test [1–4]. In particular, it is recommended to demonstrate the specificity and sensitivity in addition to trueness and precision [1, 5]. Moreover, any validation should consider robustness, a measure of the capacity of the analytical test to remain unaffected by small but deliberate variations in procedural parameters [4]. Typical variables in a laboratory of anatomic pathology include delay to fixation, fixation time, fixative type, pretreatment processing (e.g. antigen retrieval), section thickness, antigen stability, reagent stability and environmental conditions. Last but not least, a full validation is required for "in-house" or "home-brewed" tests [1–5].

Beside test optimization, it is useful to establish a validation plan before starting test validation. Initially, the equipment and the reagents to be used for the validation of the analytical test should be determined. Secondly, one should define which performance characteristics (e.g. trueness, precision, robustness, etc.) have to be verified in chronological order. The realization of a validation plan will result in a collection of data within a minimum amount of time which may help to reveal analytical errors early in the validation process [2]. Moreover, a validation plan should include the acceptance and rejection criteria for the validation examinations to be performed, as well as the validation set including the sample size based on the application field of the analytical test and the matrix intended to be tested in routine practice. The identification of the co-worker(s) performing the validation examinations, the pathologist(s) or specially trained staff member(s) who will have to review the stained sections or the results of the validation examinations as well as the co-worker(s) who will have to record all data in a validation report, etc. should also be part of the validation plan. If applicable, a time frame for performing the validation study can be defined. It might also be helpful to specify the needs for staff training, infrastructure modifications, software upgrades or updates [e.g. linkage to the laboratory information system (LIS)], establishing standard operating procedures, log books, maintenance schemes, etc. Generally, an appropriate validation plan may result in a time- and cost-saving performance of the analytical test validation.

In order to evaluate the implementation of test validation in Belgian anatomic pathology laboratories and to help them improving the validation and the overall quality of their tests, a study has been performed in which the standard operating procedure(s) (SOP) for test validation and one validation report from each of those laboratories were evaluated. The results of this study provide an understanding of the existing laboratory practices regarding test validation and its pitfalls. Validation procedures are intended to reasonably assure that the validation examinations are performed in a harmonized, fixed and efficient manner and as far as possible according to the existing guidelines or recommendations [1–7]. In order to guarantee traceability, all data that may influence the results of the validation examinations should be recorded in a validation report. Establishing a template for a validation report may help to record all those data in a harmonized manner. Briefly, documented procedures and validation reports are important to ensure efficient and standardized validation of tests. This leads to traceable evidence to physicians and patients of accurate diagnostic and prognostic testing in the anatomic pathology laboratory.

Methods

In the second half of 2016, in collaboration with the Commission of Anatomic Pathology, all licensed laboratories ($n=77$) were requested to provide their actual written standard operating procedure (SOP) for analytical test validation and one validation report of a validated test to Sciensano, the national competent authority regarding licensing of laboratories.

Both documents were evaluated for the presence of predefined items (Tables 1 and 2) by two scientific collaborators of Sciensano, one of which was a pathologist.

A score of 0, 0.5 or 1 was given to each evaluated item based on its presence and completeness (1: completely described, 0.5: incompletely described, 0: not described).

Since all the Belgian laboratories were involved, statistical inference was not performed. Results are presented by means of odds for having a completely described item (score 1) versus an incomplete or absent description (scores 0.5 and 0) on the one hand, and for having a partially or completely described item (scores 1 and 0.5) versus an absent description (score 0) on the other hand. The odds for having a completely described item versus an incompletely or absent description were calculated by dividing the number of laboratories having score 1 by the number of laboratories having score 0 or 0.5. An odds value > 1 means that there are more laboratories having completely described the particular item in comparison with the number of laboratories not having completely described it or lacked a description. The odds for having a partially or completely described item versus an absent description were calculated by dividing the number of laboratories having score 0 by the number of laboratories having a score 1 or 0.5. An odds value < 1 means that there are more laboratories having partially or

Table 1 The evaluated items related to the standard operating procedure for analytical test validation

Evaluated item	Description
Performance characteristics	A summary of all performance characteristics, with their definitions, that can be validated in an anatomic pathology laboratory: trueness, precision, sensitivity, specificity, robustness, etc
Method of validation	Presence of separate written procedures for verifying tests using CE/IVD-labeled kits or reagents, laboratory-modified tests (in the case of modification of the manufacturer's specifications) and in-house tests (self-developed or "home-brewed" tests) + a description of how and which validation examinations are performed for demonstrating each performance characteristic (e.g. trueness, precision, sensitivity, specificity, robustness, etc)
Repetition rate of the validation examinations and sample size	Description of the minimum number of validation samples that should be used to verify performance characteristics and the repetition rate of the validation examinations to be performed (e.g. intermediate precision)
Co-workers involved	Description of which co-workers perform the validation examinations, which co-workers evaluate the stained sections and/or results and formulate the conclusions (the pathologist or other trained staff members) and/or which co-workers record all the data in a validation report
Recording and archiving of results and raw data	Description of where the results and raw data of the validation examinations are recorded and archived and where the validation reports are preserved
Content of the validation report	Description of the content of a validation report (e.g., purpose of the validation, required equipment and reagents, matrix, acceptance/rejection criteria, results and evaluation, final conclusion, release, etc)
Revalidation	Description of the most common reasons for revalidation (e.g. new reagent lot, change in antibody clone, change in vendor, equipment maintenance, reparation of defective equipment, upgrade or update of computerized systems, etc.) and the method applied (e.g. determination of the performance characteristics and the minimum sample size/number)
Ongoing validation	Description of the procedure for ongoing validation: "external quality assessment" (EQA), "internal quality control" (IQC), interpersonal tuning, population study, etc
Release	A description of when ("Do all the predefined criteria have to be met for all the validation examinations performed before an analytical test can be implemented into daily routine? Can an analytical test and its validation report be released under specific conditions if not all the predefined acceptance criteria are met? What actions are taken if not all criteria are met? What are the conditions defining that a validation report cannot be released?"), how (e.g. electronic approval indicating the date or manually using a dated signature) and by whom an analytical test and its validation report are released
Implementation into daily routine	The steps that have to be undertaken to implement an analytical test taking into account staff training, establishing standard operating procedures, log books, maintenance schemes, updating the laboratory information system (LIS), etc

completely described the particular item in comparison with the number of laboratories lacking a description. For every evaluated item, the odds ratio of ISO 15189:2012 accredited laboratories to non-accredited laboratories was calculated.

Results

Written standard operating procedure for analytical test validation

The standard operating procedure for analytical test validation of each of the 77 laboratories was evaluated for the presence of predefined items as shown in Table 1.

Separate validation procedures for CE/IVD-labeled, laboratory-modified and laboratory-developed tests and a description of how each performance characteristic was validated were lacking in 51 (66 %) laboratories as shown in Fig. 1. However, a small proportion of laboratories, in particular 9 (12 %) and 10 (13 %) laboratories, had separate written procedures for predictive and non-predictive immunohistochemical tests and for qualitative and semiquantitative tests, respectively (results not shown). Eighteen laboratories (23 %) specified the minimum number of validation samples that should be used to verify performance characteristics and the repetition rate of the validation examinations to be performed (e.g. intermediate precision). However, a wide variation in the minimum number of validation samples was noticed in the procedures of the laboratories, ranging from 1 to 40 samples. In addition, only 9 (12 %) laboratories reported to have written procedures for when and how analytical tests need to be revalidated. Better results were observed regarding the description of the performance characteristics, the co-workers involved, recording and archiving results and raw data, the content of the validation report,

Table 2 The evaluated items related to the validation report of an analytical test

Evaluated item	Question
Purpose of the validation	A description of whether the validation examinations were performed in the context of an initial validation, a historical validation of an already existing analytical test, a revalidation or for any other reason
Application field of the test	A description of the intended use of the analytical test and an explanation of the function of the target to be detected and the clinical impact for the patient
Required equipment and reagents	A description of the equipment used for the validation and information about the reagents (antibody clone, probe, vendor, lot number, etc.)
Performance characteristics determined	Demonstration of the essential performance characteristics such as trueness, precision, robustness, etc.
Step-by-step instructions for performing the examinations	A description of the step-by-step instructions for each validation examination
Matrix	A description of the matrix on which the analytical test applies in routine practice, including details of the origin of the tissue, tissue fixation and processing
Sample selection	A description of the validation set: origin of tissue or cytological specimens, sample numbers, expected expression or amplification levels
Sample size	A clear determination of the sample size with a sufficient number of samples and consistently indicated for each validation examination
Acceptance/rejection criteria	Clear and objective acceptance criteria, consistently defined for each validation examination
Co-workers involved	Mentioning of the name(s) of the technician(s) that performed the validation examinations and the name(s) of the pathologist(s) or the trained staff member(s) that reviewed the stained sections or the results from each validation examination
Dates of performance	The dates of performance, consistently mentioned for each validation examination
Results obtained	A clear overview of the results obtained for each slide or run and/or reference to the raw data as well as an interim conclusion for each verified performance characteristic
Final conclusion	A general final conclusion, based on the results obtained from the validation examinations performed and taking into account the predefined acceptance criteria
Release	Clearly mentioning of the release date of the validation report and the identity of the person authorizing its release
Ongoing validation	An overview of the results obtained and/or a reference to the document and its location that contain the results of the ongoing validation

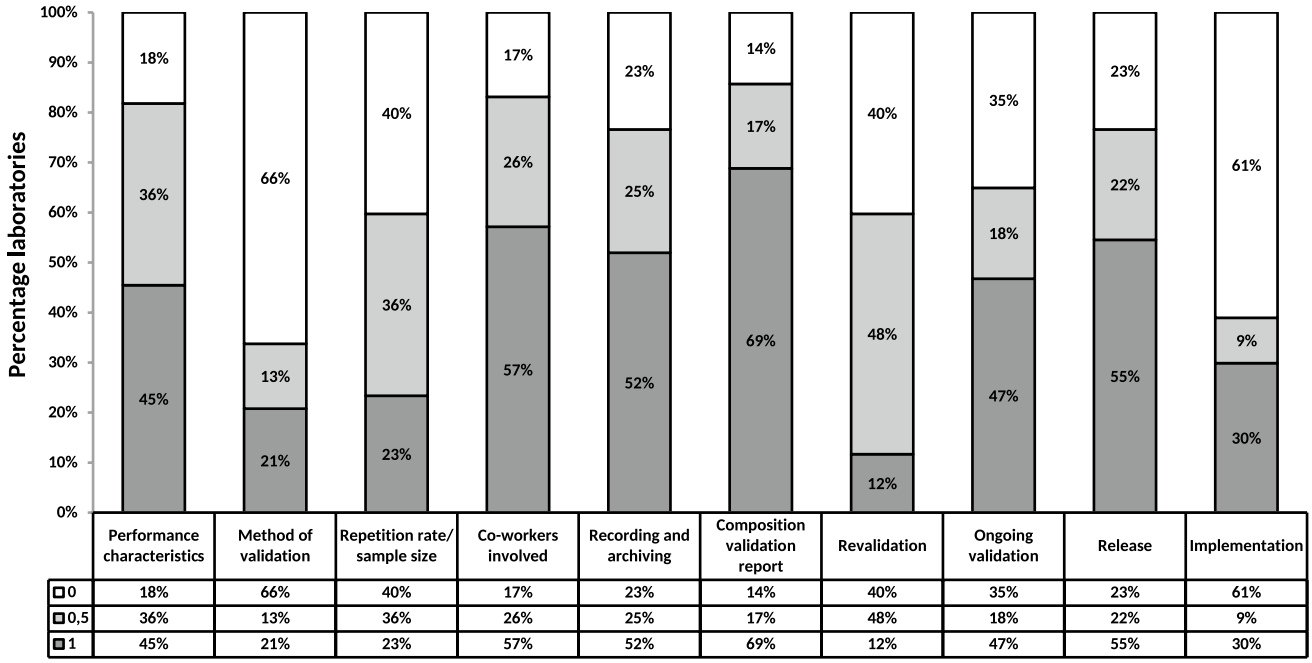


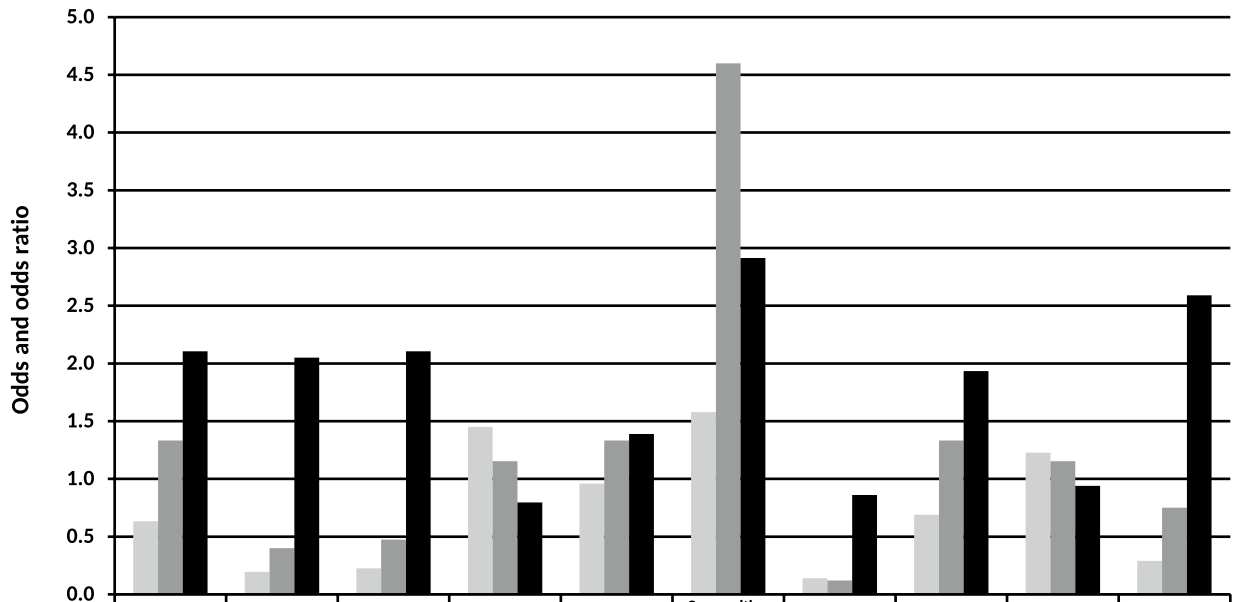
Fig. 1 Percentage of laboratories ($n=77$) scoring 0 (item not described; white bar), scoring 0.5 (item incompletely described; light gray bar) and scoring 1 (item completely described; dark gray bar) for each evaluated item in the standard operating procedure for analytical test validation

ongoing validation, release and implementation into daily routine (Fig. 1).

In Belgium, an ISO 15189:2012 accreditation on top of having a license is required for reimbursement of molecular diagnostic tests. The verification and validation of each test are a formal requirement of the ISO 15189:2012 standard. In Belgium, 28 (36 %) laboratories of anatomic pathology are accredited according to this ISO standard.

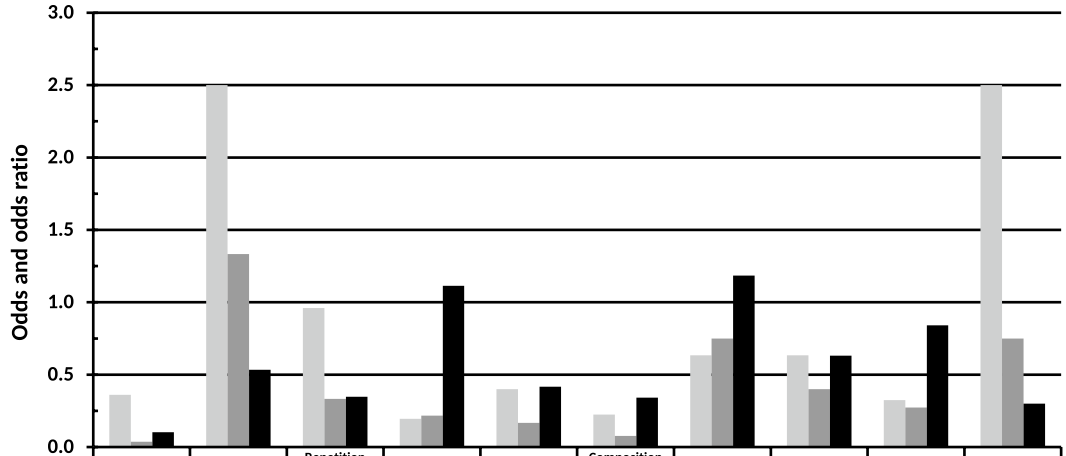
Figure 2 shows the odds and odds ratios of accredited laboratories compared to non-accredited laboratories for the presence of a complete description of the evaluated items in comparison with an incomplete description and absence. For example, for each non-accredited laboratory having a complete description of the performance characteristics, there are 1.6 (1/0.63) laboratories having an incomplete or absent description of the particular item. Higher odds values for having completely described the evaluated items "description of the performance characteristics," "the method of validation," "the repetition rate of the validation examinations and the sample size," "recording and archiving of results and raw data," "the content of the validation report," "ongoing validation" and "implementation into routine practice" were observed in accredited laboratories compared with non-accredited laboratories which resulted in an odds ratio > 1 (Fig. 2). In other words, accredited laboratories described more frequently the previously mentioned items completely. For example, a complete description of the performance characteristics was two times more frequently observed in accredited laboratories compared with non-accredited laboratories (odds ratio A/NA = 2.11, Fig. 2).

Figure 3 shows the odds and the odds ratios of accredited laboratories compared to non-accredited laboratories for not having described the evaluated items in test validation procedures in comparison with a partially or complete description. For example, for each non-accredited laboratory that lacks a description of the performance characteristics, there are 2.8 (1/0.36) laboratories having a partially or completely description of the particular item. Lower odds values for not having described the evaluated items "description of the performance characteristics," "the method of validation," "the repetition rate of the validation examinations and the sample size," "recording and archiving of results and raw data," "the content of the validation report," "ongoing validation," "release" and "implementation into routine practice" were observed in accredited laboratories compared with non-accredited laboratories which resulted in an odds ratio < 1



	Performance characteristics	Method of validation	Repetition rate/ sample size	Co-workers involved	Recording and archiving	Composition validation report	Revalidation	Ongoing validation	Release	Implementation
Odds not accredited (NA)	0.63	0.20	0.23	1.45	0.96	1.58	0.14	0.69	1.23	0.29
Odds accredited (A)	1.33	0.40	0.47	1.15	1.33	4.60	0.12	1.33	1.15	0.75
Odds ratio A/NA	2.11	2.05	2.11	0.80	1.39	2.91	0.86	1.93	0.94	2.59

Fig. 2 Odds and odds ratios of accredited laboratories (A) compared to non-accredited laboratories (NA) for the presence of a complete description (score 1) of the evaluated items in the standard operating procedure for analytical test validation in comparison with an incomplete description and absence (scores 0.5 and 0). The odds values of non-accredited and accredited laboratories are shown in the light gray and dark gray bars, respectively. An odds value > 1 means that there are more laboratories having completely described the particular item in comparison with the number of laboratories not having completely described it or lacked a description. The odds ratios A/NA are shown in the black bars



	Performance characteristics	Method of validation	Repetition rate/ sample size	Co-workers involved	Recording and archiving	Composition validation report	Revalidation	Ongoing validation	Release	Implementation
Odds not accredited (NA)	0.36	2.50	0.96	0.20	0.40	0.23	0.63	0.63	0.32	2.5
Odds accredited (A)	0.04	1.33	0.33	0.22	0.17	0.08	0.75	0.40	0.27	0.75
Odds ratio A/NA	0.10	0.53	0.35	1.11	0.42	0.34	1.18	0.63	0.84	0.30

Fig. 3 Odds and odds ratios of accredited laboratories (A) compared to non-accredited laboratories (NA) for not having described the item (score 0) versus a partially or completely described evaluated item (scores 1 and 0.5) in the standard operating procedure for analytical test validation. The odds values of non-accredited and accredited laboratories are shown in the light gray and dark gray bars, respectively. An odds value < 1 means that there are more laboratories having partially or completely described the particular item in comparison with the number of laboratories lacking a description. The odds ratios A/NA are shown in the black bars

(Fig. 3). In other words, a lack of the previously mentioned items was more frequently observed in non-accredited laboratories than in accredited laboratories. For example, a lack of description of the performance characteristics was nine times more frequently observed in non-accredited laboratories compared with accredited laboratories (odds ratio A/NA = 0.10, Fig. 3).

Validation report of an analytical test

A total of 76 validation reports were received, including eight reports of a basic technique in anatomic pathology (e.g. tissue processing), 10 of a basic or special staining method, 34 of an immunohistochemical method, 15 of a molecular diagnostic test, one of a next-generation sequencing (NGS) test, seven equipment validation reports and one market study. One laboratory did not provide a validation report. Consequently, a score 0 was assigned to this laboratory for each evaluated item.

All validation reports were evaluated for the presence of predefined items as shown in Table 2.

Contrary to the evaluation results of the procedures for test validation, better results for the content of the validation reports were obtained.

The essential performance characteristics such as trueness, precision and robustness have been demonstrated by 38 laboratories (49 %) and were clearly described in their validation reports. Additionally, the methods laboratories used to demonstrate the trueness of their analytical test were subject to evaluation. Correlation with morphology and expected results was the most common method for verifying trueness of the analytical test (48 %), followed by direct comparison with previously validated test results, either performed in another laboratory with the same test or in-house with another validated method (34 %). Comparison with the results from a formal proficiency testing program was the third most common method in demonstrating trueness of the analytical test (27 %). However, we noted that many laboratories apply multiple methods to demonstrate the trueness of an analytical test. In particular, 2 (3 %) and 22 laboratories (29 %) applied all three and two of the three above-mentioned methods, respectively. Thirty-two laboratories (42 %) demonstrated the trueness by applying only one method of which 23 laboratories only by correlation with morphology and expected results, 5 laboratories only by comparison with previously validated test results of the same test in another laboratory or another method and 4 laboratories only by comparison with the results from a proficiency testing program. Other methods for demonstrating the trueness (e.g. comparison with validated test results of another equipment but same method), whether or not in combination with one of the other above mentioned methods, were applied by 2 laboratories (0.02 %). When the trueness of the analytical test could not be sufficiently assured (for example if too few samples were examined), but precision examinations were performed, a score of 0.5 was assigned. This was the case in 13 laboratories. In total, a score of 0.5 was given to 26 laboratories (34 %, Fig. 4) of which 13 laboratories for not sufficiently demonstrating the trueness of the analytical

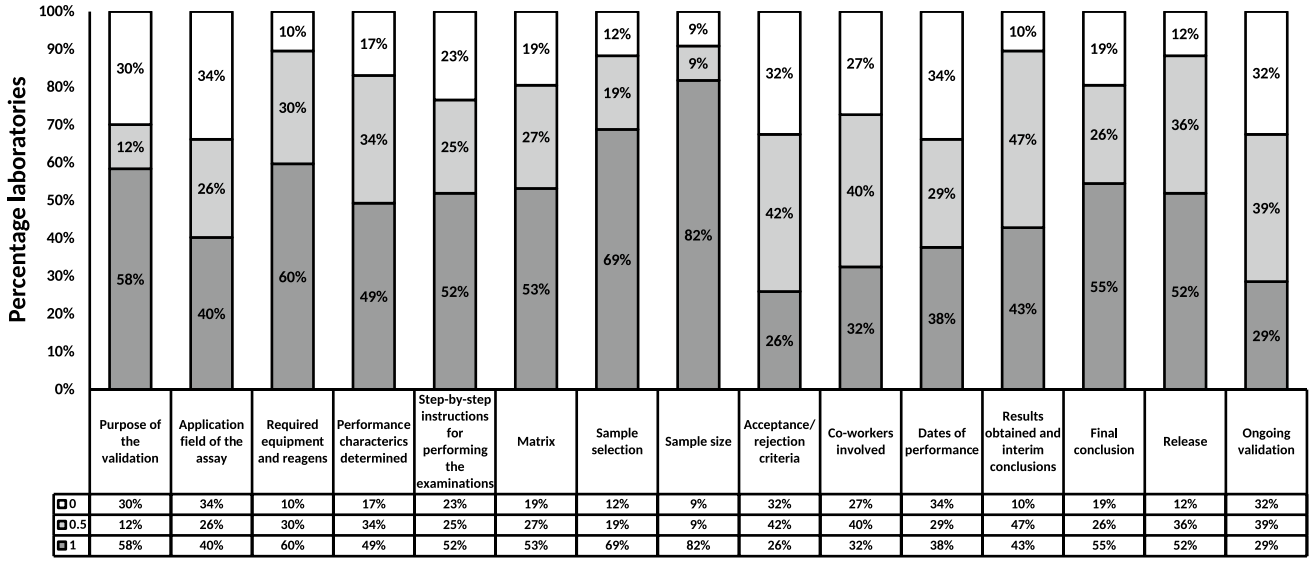


Fig. 4 Percentage of laboratories ($n=77$) scoring 0 (item not described or elaborated; white bar), scoring 0.5 (item incompletely described or elaborated; light gray bar) and scoring 1 (item completely described or elaborated; dark gray bar) for each evaluated item in the validation report

test, 12 laboratories for not demonstrating the precision and 11 laboratories for not indicating which performance characteristics were verified. Nineteen laboratories (25 %) did not demonstrate the trueness or a demonstration of the trueness was not applicable in their validation report (e.g. equipment validation or validation of a basic technique). A score of zero was assigned if the performance characteristics to be verified were not indicated, if no precision tests were performed and if the trueness of the analytical test could not be guaranteed. This was the case in 13 laboratories (17 %, Fig. 4).

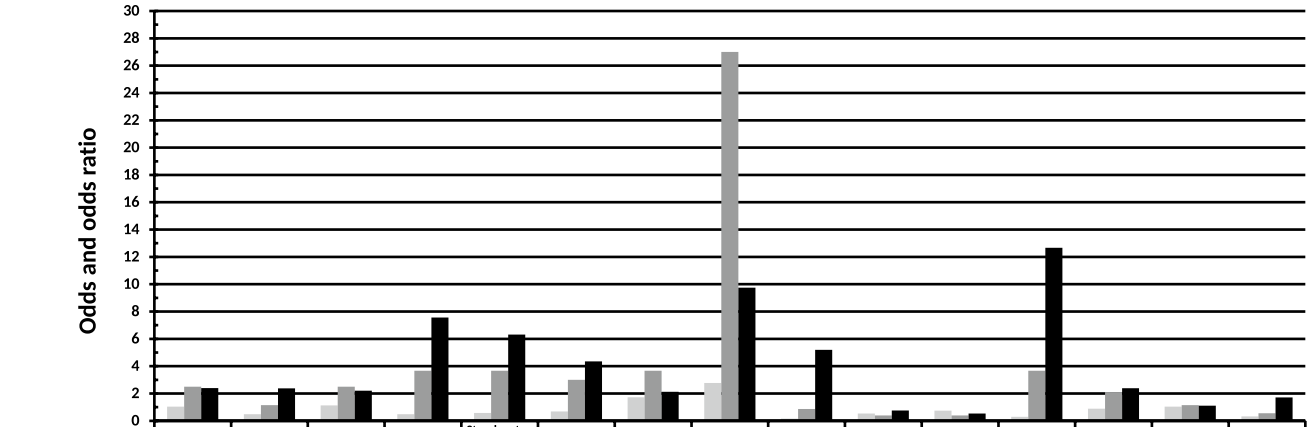
Although the international ISO 15189:2012 standard provides very little guidance about specific requirements and procedures for verifying and validating analytical tests, a complete description of all items evaluated was seen more frequently than an incomplete description or absence (except for the co-workers involved and the dates of performance) in accredited laboratories in comparison with non-accredited laboratories (Fig. 5). For example, a complete description of all essential performance characteristics was seven to eight times more frequently observed in accredited laboratories compared with non-accredited laboratories (odds ratio A/NA = 7.56, Fig. 5).

Nevertheless, the identification of the co-workers involved was two times more frequently missing in the validation report of accredited laboratories compared with non-accredited laboratories (odds ratio A/NA = 1.92, Fig. 6).

Discussion

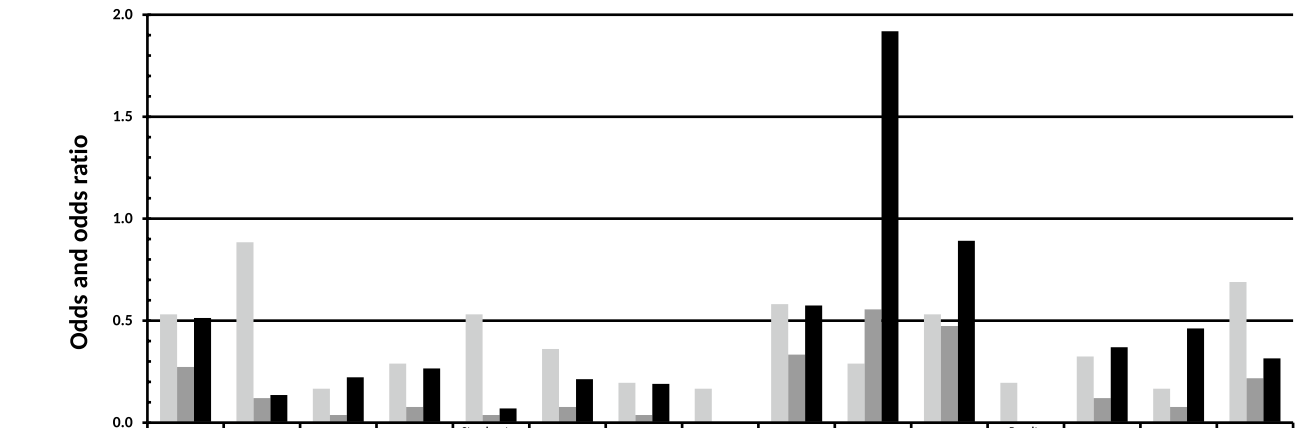
This study highlights some particular issues regarding validation of tests in Belgian anatomic pathology laboratories. Only one-fifth had written standard operating procedures regarding the method applied for revalidating analytical tests, and less than a third had separate validation procedures for CE/IVD-labeled tests, laboratory-modified tests and "home-brewed" tests. Better results regarding the presence of written procedures for test revalidation were reported in the study performed by Stuart et al. [8] in which 61.4 % of the laboratories reported having written procedures for revalidation of immunohistochemical tests for both non-predictive and predictive markers whereas 3.7 % reported having procedures for predictive markers only and 8.4 % for non-predictive markers only. Moreover, having separate written procedures for CE/IVD-labeled tests was reported by 54.2 % laboratories [8]. The results of our study and the results of the study of Stuart et al. demonstrate the need for guidelines and recommendations regarding validation and revalidation of analytical tests in laboratories of anatomic pathology.

Having separate written procedures for predictive and non-predictive markers is becoming increasingly important since the introduction and the increase of personalized medical treatments. This study demonstrates that a only 12 % of the laboratories have separate written procedures for predictive and non-predictive markers compared with 42.7 % of the laboratories in the study of Stuart et al. [8]. However, specific recommendations for initial analytical validation and revalidation of predictive and non-predictive marker tests have been published by Fitzgibbons et al. in 2014 [1]. Particularly, this paper provides recommendations for parameters included in the validation set, such as the minimum sample size, expression levels, fixative and processing methods. In this study, 34 laboratories submitted a validation report of an immunohistochemical test. Only



	Purpose of the validation	Application of the assay	Required equipment and reagens	Performance characterics determined	Step-by-step instructions for performing the examinations	Matrix	Sample selection	Sample size	Acceptance/ rejection criteria	Co-workers involved	Dates of performance	Results obtained and interim conclusions	Final conclusion	Release	Ongoing validation
Odds not accredited (NA)	1.04	0.48	1.13	0.48	0.58	0.69	1.72	2.77	0.17	0.53	0.75	0.29	0.88	1.04	0.32
Odds accredited (A)	2.50	1.15	2.50	3.67	3.67	3.00	3.67	27.00	0.87	0.40	0.40	3.67	2.11	1.15	0.56
Odds ratio A/NA	2.40	2.38	2.21	7.56	6.31	4.35	2.13	9.75	5.20	0.75	0.53	12.67	2.39	1.11	1.71

Fig. 5 Odds and odds ratios of accredited laboratories (A) compared to non-accredited laboratories (NA) for the presence of a complete description (score 1) of the evaluated items in the validation report in comparison with an incomplete description and absence (scores 0.5 and 0). The odds values of non-accredited and accredited laboratories are shown in the light gray and dark gray bars, respectively. An odds value > 1 means that there are more laboratories having completely described the particular item in comparison with the number of laboratories not having completely described it or lacked a description. The odds ratios A/NA are shown in the black bars



	Purpose of the validation	Application field of the assay	Required equipment and reagens	Performance characterics determined	Step-by-step instructions for performing the examinations	Matrix	Sample selection	Sample size	Acceptance/ rejection criteria	Co-workers involved	Dates of performance	Results obtained and interim conclusions	Final conclusion	Release	Ongoing validation
Odds not accredited (NA)	0.53	0.88	0.17	0.29	0.53	0.36	0.20	0.17	0.58	0.29	0.53	0.20	0.32	0.17	0.69
Odds accredited (A)	0.27	0.12	0.04	0.08	0.04	0.08	0.04	0.00	0.33	0.56	0.47	0.00	0.12	0.08	0.22
Odds ratio A/NA	0.51	0.14	0.22	0.27	0.07	0.21	0.19	0.00	0.57	1.92	0.89	0.00	0.37	0.46	0.32

Fig. 6 Odds and odds ratios of accredited laboratories (A) compared to non-accredited laboratories (NA) for not having described the item (score 0) versus a partially or completely described evaluated item (scores 1 and 0.5) in the validation report. The odds values of non-accredited and accredited laboratories are shown in the light gray and dark gray bars, respectively. An odds value < 1 means that there are more laboratories having partially or completely described the particular item in comparison with the number of laboratories lacking a description. The odds ratios A/NA are shown in the black bars

three of them (9 %) apply the recommendations of Fitzgibbons et al. Moreover, we noticed a wide variation in the number of samples used for the validation of the analytical test in the validation reports, ranging from 1 to 50 samples. Globally considered, the results of this survey suggest that the recommendations of Fitzgibbons et al. are probably not well-known or implemented in the Belgian laboratories of anatomic pathology. Although, taking into account the increase in the development of new technologies and therapeutic drugs, laboratories will be forced to elaborate separate written procedures for validating predictive markers of immunohistochemical tests.

Demonstration of the trueness for each new analytical test before being implemented into daily practice is generally accepted. The different methods allowing the anatomic pathology laboratories to demonstrate the trueness of an immunohistochemical test have been described by Fitzgibbons et al. [1]. However, one may question the necessity of verifying the precision of each analytical test including each non-predictive or predictive test when the same technique and equipment are used. In such cases, a more restricted validation with demonstration of the trueness of the analytical test and reference to the results of the verification of the precision and robustness in the validation report of the equipment used or the equivalent analytical test using the same technique and equipment should be sufficient. In this study, 25 Belgian laboratories of anatomic pathology reported not verifying the precision of the analytical test nor referring to the results of the precision verification within the framework of the validation of the equipment or the equivalent analytical test. In summary, verification of performance characteristics such as precision, robustness, specificity and sensitivity in addition to trueness depends on the analytical test to be validated and its intended purpose. The decision for not verifying one or more performance characteristic(s) contrary to what the standard operating procedure prescribes should be motivated in the validation plan or report. Performing a risk analysis, taking into account the intended use of the analytical test, may be an efficient tool to endorse this decision [9–11].

Procedures for equipment validation and test validation may interrelate and overlap. Equipment validation consists of three major steps: installation qualification, operational qualification and performance qualification. In the latter step, one verifies whether the equipment functions correctly and consistently for the intended application. Therefore, laboratories have to demonstrate the repeatability and the intermediate precision of the equipment. As a consequence, a laboratory may refer to the precision of the equipment when verifying this performance characteristic of the analytical test for which the equipment is used. Depending on the analytical test for which the equipment has to be validated, all data of the validation process can be recorded into the validation report of the equipment. Examples include equipment for mounting slides, embedding cytological specimens, etc. In this survey, seven laboratories submitted equipment validation reports including the validation of the analytical test.

In addition to defining the equipment and the reagents used for the validation of the analytical test and the performance characteristics to be demonstrated, the third most important parameter to be defined before starting test validation is the criteria for accepting or rejecting the analytical test. The results obtained from a validation study have to meet the predefined, objective acceptance criteria before the new analytical test can be released and implemented into daily practice. A lack of clear and predefined objective acceptance criteria for each performance characteristic in the validation report of the analytical test was the most common shortcoming observed in the Belgian laboratories of anatomic pathology (Fig. 4). In some validation reports, objective acceptance criteria were lacking. It would be better to describe the criteria such as “correct,” “good,” “adequate” and “sufficient” in an objective manner, for example, “no cracks” (when verifying the quality of tissue sectioning), “no air bubbles” (when verifying the quality of mounting tissue sections), etc. It can be useful to apply the SMART (specific measurable achievable relevant and time-bound)-principle in determining objective acceptance criteria. In addition, they must be defined considering the intended use of the analytical test. In the case of a concordance study, for instance, laboratories should achieve at least 90 % or 95 % overall concordance for, respectively, non-predictive and predictive biomarker tests, between the new test results and the results of the previously validated analytical test or another validated analytical test [1]. As for the verification of the specificity and sensitivity, the objective acceptance criteria may consist of a clear description of the tissue and cell structures that should stain as well as the staining pattern. To demonstrate precision, objective acceptance criteria may consist of a minimum percentage of concordant results. It should be pointed out that the concordance achieved (in the case of accuracy tests, precision tests, etc.) depends on the number of samples tested. The larger the validation set, the higher the concordance percentage that can be achieved [1, 4]. Additionally, a literature study may be useful, for example in the case of analytical tests related to less frequent diseases, to determine the acceptance criteria.

Also defining the validation set such as the origin of tissue or cytological specimens, the minimum sample size, the expression or amplification levels, the fixation and processing methods is crucial before starting test validation. The most appropriate selection of tissues in a validation set depends on the matrix intended to be used in the routine diagnostic laboratory. The matrix of the validation materials must be representative for the patient specimens [1, 3]. Known positive and negative samples previously tested by a validated laboratory method (either within the laboratory or by another laboratory), reference standards, cell lines and excess tissue previously used in an external quality assessment or in an interlaboratory comparison program may be selected for test validation [1, 2]. However, matrix-induced effects, including specimen origin, fixative type, fixation time, tissue processing, antigen retrieval method, etc., have to be taken into consideration as they may not be representative for the specimens received in routine practice and may influence the test results and conclusions [2, 3, 8]. Therefore, it is recommended to select as many patient specimens as possible in addition to samples from other sources [2].

In this study, a complete description of the matrix was present in the validation report of 41 laboratories of anatomic pathology (53 %, Fig. 4). Furthermore, a description of the validation set used to perform the validation examinations was sufficiently detailed in more than two-third of the validation reports (Fig. 4). We recommend mentioning for each sample specimen in the validation set the specimen origin, the sample number and the expected expression or amplification level of the target in order to trace back to the matrix to which the analytical test applies. The validation set should include high and low expressors for positive cases (e.g. for immunohistochemical tests) and should cover the expected range of clinical results (cfr. semiquantitative and quantitative tests) [1, 4]. Therefore, tissue microarrays (TMAs) and multitissue blocks (MTBs) can be used [1, 7].

The minimum sample size of the validation set depends on the analytical test to be validated. This study showed that more than three quarters of the laboratories of anatomic pathology did not establish in their standard operating procedure for analytic test validation the minimum number of validation cases that should be used to verify the predefined performance characteristics. However, key criteria to determine the number of samples needed to validate immunohistochemical methods have been published by Fitzgibbons et al. [1]. Nevertheless, the sample size may be limited by sample availability and budget concerns [1, 2, 4]. Interlaboratory cooperation to collect a suitable number of samples may provide a solution to attain the required power for a representative validation [1, 4]. Despite the recommendations of Fitzgibbons et al. regarding the minimum number of samples needed for initial validation of immunohistochemical methods, guidelines to determine the minimum sample size for validating other analytical methods performed by anatomic pathology laboratories such as special staining methods in histology are lacking. In this study, a small number of laboratories reported using only one sample to demonstrate the performance characteristics of the analytical test. However, we recommend using more than one sample for validating basic and special staining methods. Furthermore, Fitzgibbons et al. did not clearly define to which performance characteristics to be demonstrated in the validation test the recommendations have to be applied. Most probably, they have to be applied to demonstrate the trueness, specificity and sensitivity. For demonstrating the precision, it is recommended either for qualitative and semiquantitative methods to test at least one positive specimen, one weakly positive specimen when appropriate and one negative specimen in triplicate [3, 5, 7]. Finally, performing a risk analysis can be a helpful tool in determining either the minimum sample size for validating the analytical test as well as the potential sources of variations in the analytical test.

Test validation is an ongoing process. Revalidation is required if the existing validated test is modified. The method for revalidating analytical tests depends on the test itself. Recommendations for revalidation of immunohistochemical methods have been published by Fitzgibbons et al. [1]. However, recommendations for revalidation of other analytical tests performed by laboratories of anatomic pathology are lacking. Performing a risk analysis may help to determine the performance characteristics to be redemonstrated and the parameters in the validation set including sample size.

In summary, this study shows a lack of standardization of verification and validation procedures among the Belgian anatomic pathology laboratories. Even in accredited laboratories, disturbing results of compliance to ISO 15189:2012 were observed as shown in Figs. 2, 3, 5 and 6. This outcome implies the need for univocal and standardized instructions for specific fields of laboratory medicine in order to meet the requirements of this standard. At the Belgian level, the Commission of Anatomic Pathology is authorized to establish specific instructions, requirements and points of attention for analytical test validation, as specified in Tables 1 and 2, into the practical guideline. This will also force the Belgian accreditation body (BELAC) and its auditors to adapt their assessment process which will improve the compliance of the accredited laboratories to essential parts of the ISO 15189:2012 standard. Although the practical guideline only applies to Belgian laboratories, it is appropriate to elaborate international guidelines since evidence-based requirements and guidelines regarding validation of tests in laboratories of anatomic pathology are scarce. In particular, practical guidelines for the complete process of optimization and validation of histological, immunohistochemical and molecular diagnostic tests as well as next-generation sequencing-based oncology panels are limited [1, 4, 6, 12]. Hence, establishing international guidelines and standardization for validating and revalidating tests in laboratories of anatomic pathology are important in reducing potential harms of false positive and false negative results, which may cause under- or overtreatment of patients and cost-ineffective patient care worldwide.

Finally yet importantly, we encourage our colleagues within Belgium and from other countries to perform similar compliance investigations to ISO 15189:2012 in laboratories for clinical biology and other fields of laboratory medicine (e.g. genetics) as well as to study the compliance to other specific requirements of this standard besides validation and verification of analytical tests.

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