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Review

Validation conform ISO-15189 of assays in the field of autoimmunity: Joint efforts in The Netherlands☆

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ABSTRACT

ISO 15189:2012 requires validation of methods used in the medical laboratory, and lists a series of performance parameters for consideration to include. Although these performance parameters are feasible for clinical chemistry analytes, application in the validation of autoimmunity tests is a challenge. Lack of gold standards or reference methods in combination with the scarcity of well-defined diagnostic samples of patients with rare diseases make validation of new assays difficult. The present manuscript describes the initiative of Dutch medical immunology laboratory specialists to combine efforts and perform multi-center validation studies of new assays in the field of autoimmunity. Validation data and reports are made available to interested Dutch laboratory specialists.

Contents

1. Introduction

In Europe the Standard ISO 15189:2012 "Medical Laboratories – Requirements for quality and competence" (ISO15189) has been adopted as accreditation standard for the medical laboratories. This norm is a general standard and does not include specific standards or requirements for highly specialized parts of clinical laboratories, for instance the autoimmune laboratory. Recently an ad hoc committee of the European Standardization Initiative (EASI) provided background information on accreditation for autoimmune laboratories and described some of the problems an autoimmune laboratory can encounter in the accreditation process [1]. In addition they challenged EASI in providing recommendations for both accreditation bodies and clinical laboratories when

Abbreviations: EASI, European Standardization Initiative; CMI, College of Medical Immunology; SSc, systemic sclerosis.

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assessing competencies in the field of autoimmunity. One of the suggested efforts they mention for EASI is an advice on how to validate autoantibody tests and recommendation of harmonized validated and well-documented approaches.

Validation of autoimmunity tests is indeed a challenge. The performance parameters for validation mentioned in ISO 15189 (trueness, uncertainty, measurement interval, linearity, precision, accuracy, limit of detection, analytical and diagnostic sensitivity and specificity) are more easy to determine for clinical chemistry analytes than for autoantibodies. For the detection of autoantibodies gold standards, reference methods and reference materials usually are not available. In addition, despite many national and international efforts [2,3], harmonization of autoantibody tests is still a long way to go. At the same time the number of detectable antibodies is increasing steadily, which are subsequently incorporated into diagnostic and classification criteria of often rare autoimmune diseases.

In preparing validation plans of autoantibody tests, individual laboratories encounter problems like lack of gold standards and lack of sufficient diagnostic patient samples of relevant disease cohorts. It is well known that antibodies in the course of disease can alter, for instance by affinity maturation or epitope spreading. Since most antibody tests are used for diagnosis or supporting diagnosis it is therefore important that validation of clinical characteristics of autoantibody tests is ideally performed with diagnostic samples. As a consequence most validation studies performed by individual laboratories consist of method comparison rather than clinical evaluation.

Autoantibody profiles can differ in different ethnic backgrounds. Multi-component diagnostic tests, for instance line blots, may contain antigens that are not common for the local patient population. In study cohorts for validation it can be hard to obtain valid data about these components, as is illustrated for the fibrillarin antigen in the multiparameter line blot for scleroderma in the study of Bonroy et al. [4].

In the Netherlands most clinical laboratories are at this moment in transition for, or already working according to accreditation against the international ISO15189 standard. These laboratories include specialized laboratories for medical immunology. Dutch medical immunologists are associated in the College of Medical Immunology (CMI) of the Dutch Association for Immunology [5]. To encounter the difficulties around validation of autoimmunity tests the CMI installed a working group Validation. The assignment for the Working Group was initially to prepare a practice guideline for validation of autoimmunity tests as supporting tool for individual laboratories. Gradually the assignment evolved into an initiative to combine efforts in autoimmunity test validation: multicenter validation with sufficient well-defined and appropriate patients samples in order to improve the quality of the validation studies, sufficing ISO standards. Additional benefit is that the documented information from multicenter studies enables the individual laboratory to perform only a method verification which is a more achievable goal. Exhibit 1 explains the difference between method validation and method verification.

Exhibit 1
Test method validation versus verification (Adapted from [6]).

Test method validation is demonstration via objective evidence that a **new or modified** examination procedure is appropriate for **a specific intended use** in medical diagnostics, and that it complies with the relevant acceptance criteria as described by the medical laboratory. This requires the experimental characterisation of all relevant performance characteristics.

Test method verification is confirmation via objective evidence that an **already validated** examination procedure is appropriate for a specific intended use in medical diagnostics in one's own working environment (laboratory), and that it complies with the acceptance criteria as described by the medical laboratory. Once performance characteristics are known and documented, the verification whether they meet local criteria under local conditions requires less experimental data and for some characteristics verification may be even achieved without the production of new data.

This means that objectively obtained performance characteristics that are not influenced by the working environment and that are documented in the validation report do not need to be verified again in the individual laboratory. Performance criteria that can be influenced by the working environment need to be verified. In the verification report the laboratory has to document for every performance characteristic whether it was verified to meet local acceptance criteria by experimental acquisition of data or by a documented rationale. For those performance characteristics that are dependent of local variables such as instrument pipetting precision, it is obvious that verification requires local data acquisition. Other characteristics such as clinical sensitivity or analytical specificity are more likely to be independent of local variables and may be verified without local experiments.

2. Multicenter validation studies – first experiences

In the first phase activities of the CMI Working Group were focused on the compilation of a practice guide in which the process of method validation and the requirements were defined according to ISO15189. In Exhibit 2 the requirements for validation of autoimmune tests according to the Working Group are depicted.

In the second phase the CMI Working Group initiated a multicenter validation study of a new automated assay for anti-Scl70 antibodies (anti-Scl70^s test, ThermoFisher) together with three medical immunology laboratories from large university hospitals (VUMC, LUMC and Radboudumc). The combined effort resulted in a valuable report that was distributed among the interested users of the ThermoFisher anti-Scl70^s test. This report was especially valuable because more than 300 systemic sclerosis (SSc) patients could be included as well as a series of suspected SSc patients combined with sufficient diseased and normal controls. This enabled us to validate the claim of the manufacturer that the new test was more sensitive than the old test with equal specificity. To validate this in individual laboratories would have been difficult in respect of the size of the cohort needed for sufficient power. During this process the Working Group recognized the need for a workflow description of a multicenter validation and developed a useful strategy (Fig. 1).

Next we started a new multicenter validation study according to the CMI practice guide. In short the working group called for participants in the multicenter validation of the Siemens Thyroid Stimulating Immunoglobulin assay. Three centers were willing to participate. The validation study was planned, validation was performed and a report was written. The Working Group Validation of the CMI reviewed both the plan and the report in order to see whether it fulfilled the standards that were agreed on, monitored the progress and facilitated the distribution of the approved report among Dutch medical immunologists and interested clinical chemists.

The report contained objective information about clinical performance characteristics such as the sensitivity, specificity and positive predictive value for M. Graves as well as analytical performance characteristics such as (among others) interference, sample type, linearity and precision. In addition, correlation of the results of the new assay with three commonly used other assays was documented. The report was highly appreciated by laboratory specialists considering introducing the test. According to the manufacturer the multicenter validation report did not result in less requests of free reagent for validation/verification but they mentioned that the test was remarkably quickly operational compared to their experience with previously launched

Exhibit 2
Phased plan of the validation study.

1. Aim of the validation protocol and the intended use of the tested analyte.
2. Summary of relevant facts of the analyte (literature, data of manufacturer, biological variation, and so on)
3. Prospective risk analysis of the analysis process (identification of specifications for the assay, instrument or performance characteristics)
4. Validation protocol including argumentation of which performance characteristics are or are not being tested, including the acceptance criteria (Performance characteristics: measurement trueness; measurement accuracy; measurement precision, analytical specificity, including interfering substances; analytical sensitivity, detection limit and quantitation limit; stability; measuring interval; diagnostic specificity and diagnostic sensitivity of the measurement; method comparison; reference range)
5. Description of the number of tests to be performed (statistically substantiated)
6. Description of the selection of the patient and control samples
7. Description of the reagents and instruments needed
8. Description of responsibilities and time line.
9. Documentation of the results
10. Interpretation of the results, including testing against the acceptance criteria
11. Conclusion
    In case additional experiments have to be performed, follow step 4–11 again.

tests. This possibly indicates that only concise verification studies were performed in the individual laboratories.

3. Discussion

Multicenter validation studies instead of validation studies in each individual laboratory have the potential to improve the quality of the efforts made. ISO15189 allows verification of new assays when the laboratory specialist can demonstrate that the new assay fulfills the performance characteristics needed for its intended use. For autoantibody assays playing a role in often rare diseases, confirmation of clinical test characteristics can be difficult. The information accompanying the tests, for instance the instructions from the manufacturer, contains in most cases clinical test parameters as sensitivity or specificity but description of the samples used is often lacking and the amount of samples used is usually limited. An individual laboratory often encounters the same problems to achieve a valid cohort of patient samples.

Multicenter validation studies therefore have the potential to be of higher quality because better defined and more extended patient cohorts are incorporated and because the knowledge and expertise of the involved immunologists is shared.

In the Netherlands the Working Group Validation of the CMI started an initiative for multicenter validation of autoimmunity tests in order to share these validation results among Dutch medical immunologists. The primary goal of this initiative was the improvement of the quality of the studies, in combination with reduction of all the individual efforts made to introduce new tests in the laboratories that perform autoimmune diagnostics. Individual laboratories can introduce new tests with concise verification studies, by referring to the more extensive report produced by the Working Group, and thereby a lot of "double work" can be prevented. In addition, we anticipate that due to the improved quality of validation approach and results, together with a well-defined process that has been agreed among the professionals in the field, this collaboration will eventually lead to less discussion about method validation during ISO audits.

We started on a voluntary basis, with no financial arrangements made. The concept is to evenly distribute the efforts that are made. However, due to the fact that larger specialized laboratories in for instance university settings will more often have well-defined patient samples available than small individual laboratories, they will probably be asked more often to participate than smaller laboratories. We will have to wait and see if the no-fee-concept will stand in the future.

Another possible risk of the chosen concept is the fact that when efforts are made to validate a specific test with well-defined and sufficiently large cohorts, the assays that are NOT tested with these cohorts do not benefit. Maybe this will lead to pressure from manufacturers to incorporate their assays in these studies. In a recently started multicenter validation study we therefore made effort to incorporate the most commonly used tests for the antibody to be validated (anti-intrinsic factor) and noticed that –so far– manufacturers are willing to cooperate and support this initiative. In addition it is conceivable that laboratories will preferably select those assays that are nationally validated, resulting in an economic advantage for selected manufacturers and, as a disadvantage, resulting in a dominated market for the validated test. *It must be clear that the aim of our effort is not to state that the validated assay is superior to other assays, our aim is to state with confidence that the test is suitable for its intended use.*

The outcome of a validation study can also be unfavorable: the test does not meet the acceptance criteria for its intended use. These reports – when started as a CMI validation study – will also be available for interested medical immunologists. Since the start of our initiative we did not experience pressure from the manufacturers to withhold information. In order to assure the independence of the process, we will not allow data sharing other than the final report or publication. Furthermore, we will not comply with requests of manufacturers with respect to restrictions to publication of the results, including requests for interim results or patient materials. Nevertheless, there may be requests from the manufacturers to incorporate additional experiments in the study. Those requests will be considered.

Ideally, validation studies of new reagents are planned, performed and published by independent relevant laboratory and clinical specialists prior to launch, but in close collaboration with the manufacturer. Bossuyt et al. wrote an ethical reflection on standardization of clinical laboratory medicine in which they referred to the social responsibility of diagnostic manufacturers [7]. In this respect a parallel might be drawn toward validation of autoimmunity tests. To contribute to the safety and well-being of patients and improvement of the healthcare system, well-developed validation studies with sufficient and relevant samples of patients with rare diseases and different penetration into various ethnic backgrounds could be considered as shared responsibility of manufacturer, laboratory specialist and clinicians.

So far, we encountered positive feedback from both the medical immunologists as the manufacturers of diagnostic assays on the validation reports produced in the name of our initiative. This encouraged us to continue the chosen path and new national validation studies are running at the moment. Obviously, based on the experience of the validation studies that have been finalized, the practice guide will further evolve. Also, it is recognized that this is a national initiative, while ISO-accreditation applies for a much wider geographical area. Therefore, the optimal goals would be that upon introduction of a new test for autoantibodies, the diagnostic company already can provide an independent validation report that fulfills all the ISO requirements.

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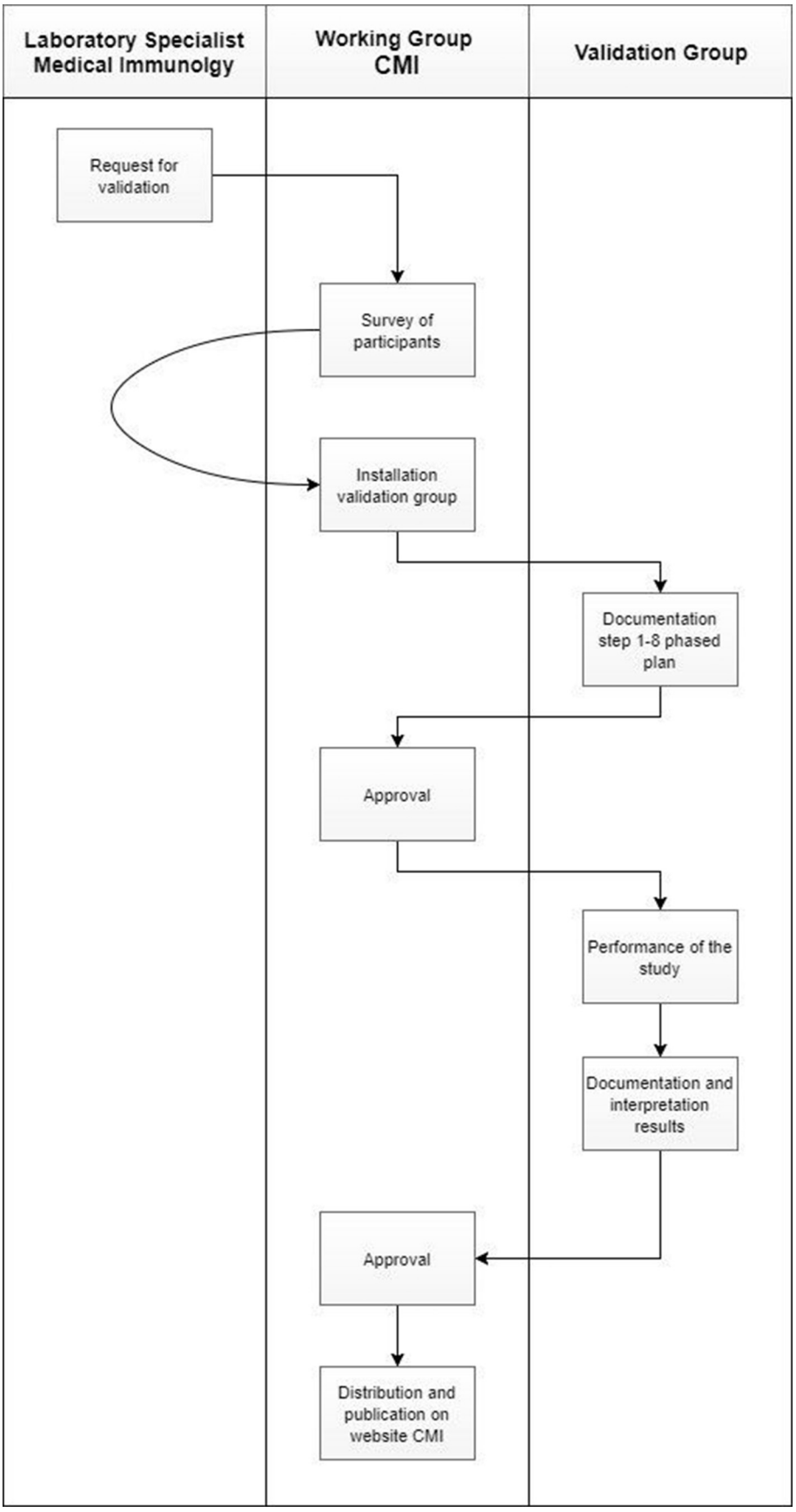

Fig. 1. Process flow.

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