METROLOGY, QUALITY, STANDARDIZATION AND CERTIFICATION

VALIDATION AND VERIFICATION OF MEASUREMENT METHODS IN CLINICAL DIAGNOSIS

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Abstract. The article investigates and analyzes the validation and verification of measurement methods in the clinical diagnostic laboratory. The content and features of validation and verification are revealed. Measurement methods are considered in detail. Each direction of validation and verification of measurement methods is analyzed. The difference between validation and verification is substantiated. Measuring systems are increasingly used in the laboratories of the clinical sector. This means that the responsibility for validation lies mainly with the manufacturer. The laboratory may operate a validated methodology, which, for example, is published as a standard, or purchase a ready-made measuring system from a manufacturer for a specific application. In both cases, the main validation operate has already been done, but the laboratory must still confirm its ability to apply the method.

Key words: Validation, Verification, Model, Measurement, Clinical diagnostics, Quality assessment.

1. Introduction

Obtaining reliable and reproducible data in a medical laboratory is possible under specific conditions of implementation of elements of the entire system ensuring the research quality [1]. Its low quality leads to negative consequences, in particular in the development of health care. The current situation is a consequence of the lack of organizational and technical principles, as well as a systematic approach to the problems of laboratory care. The issue of the quality of clinical laboratory research is relevant today in laboratory medicine and health care.

The process of verifying measurement procedures is associated with numerous data and calculations based on the statistics and probability models. To conduct a valuable "check", is necessary big data processing. Effective verification of methods is impossible without software that stores and systematizes data, computes, can conclude on the suitability of the result, including the verification of the methodology that meets the regulations [2-4].

The "Validation" software is designed to automate the calculation of the characteristics of methods during their verification (assessment of suitability). The program is developed according to the procedure of the clinical diagnostic laboratory (further - CDL) quality system. The variety of problems in the field of laboratory medicine is much wider regarding similar fields. This is due to the lack of consensus among the stakeholders concerning the patient's diagnostics and treatment. The obtained results of laboratory tests gain weight only when modern equipment and high-quality diagnostic kits are applied. The clinical laboratory is increasingly integrated with patient care, assisting in diagnosis, monitoring therapy, and prediction of clinical outcomes.

Prospects in the gradual development of validation and verification of measurement methods in the CDL are currently the subject of detailed studies. Consideration of validation and verification of CDL measurement methods becomes one of the major ones in the quality strategy [5]. Statement of the problem in the context of determining the innovative prerequisites and trends in the considered area requires detailed research.

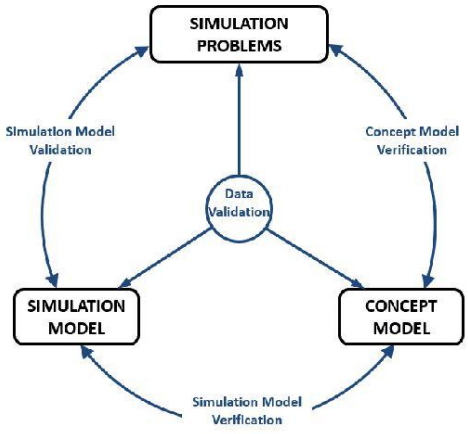

Fig. 1 Model verification and validation architecture

The emphasis of the current issue consists of methods for model verification and validation. The application of them is given in Fig. 1 as an example of the model verification and validation architecture. Here 3 stages of model verification and validation are shown. First, testing a conceptual model ensures that the conceptual model is an accurate reflection of the modeling problems identified in the real system. Second, verification of the simulation model ensures that the computer simulation model is sufficient accuracy to

represent the conceptual model. Finally, the verification of the simulation model involves a series of simulation experiments aimed at the confirmation of the needed model accuracy and efficiency to understand the particular problems. In addition, the received data concerning the model verification and validation are verified.

2. Purpose of the article

Basing on the continuous studies, assess the suitability of validation and verification of measurement methods in the clinical diagnostic laboratory practice and give examples of these operations.

3. Research in the article

Verification is the process of ensuring that a conceptual model is transformed into a computer model with sufficient accuracy or assurance of the correctness of the model. Validation, on the other hand, is the process of ensuring that a model is exact enough for the needed purpose or be persuaded in the right model design. The key concept consists in convincing everybody in providing sufficient accuracy since a model of 100 % exactness is absent.

However, in verification and validation, the goal is to make the model accurate enough. In addition, this accuracy relates to the purpose for which the model is proposed. For example, several demonstration models are quite inaccurate, and due to the sole purpose of their design, they can still be supposed as valid. Therefore, the objectives of the model must be known before it can be verified. This goal could be determined at the start of the simulation study and maybe an alternative for the existing model [6].

In the CDL practice can exist difficulties on method validation, as it is a complex process: from planning the validation to completing the results and introducing the data in the relevant documents of the conformity assessment body (for example, F - 08.01.33 / F-08.02 .33 NAAU) [7]. It should be noted that recently arise issues in CDL operation caused by the inconsistency of verification [8]. It determines the verification as confirmation by substantiation of evidence that the requirements set at the start of the appliance could be met. This concept is quite similar to the definition of validation. According to VIM [9], verification is the provision of objective evidence that an object meets the required requirements, and validation is the verification that the established requirements correspond to a specific application. VIM defines "verification" as "the provision of objective evidence that a given facility meets the established requirements" [10].

CDL can also operate based on the approved method, previously published as a standard, or purchase a specific measuring instrument from the manufacturer. In both cases, the main part of verification has been performed already. Then CDL has to confirm its ability to apply the considered method. Such confirmation is qualified as verification. It means that laboratories need to perform certain experiments to prove that the method operates properly. Consequently, the share of operate performed is smaller compared to the validation of the method fulfilled in CDL.

Currently, worldwide CDLs perform plenty of tests of measurement procedures related to healthcare needs. It is important to measure exactly and prove the correctness of the obtained results. Research that has confirmed the presence of illicit drugs may be grounds for a fine, imprisonment, etc.

The method must be validated when it is necessary to make sure that its characteristics are suitable for a particular application. For example, in [11] it is stated that CDL should confirm:

– non-standard methods;

– methods created/developed in CDL;

– standard methods in case of their operations outside the established sphere;

– extension and modification of standard methods.

The number of verifications has to be sufficient to meet the requirements specific to the considered task. The degree ("scale", "scope") of validation depends significantly on the specific use**,** the nature of the changes, and the conditions to which the method is applied. Validation is required when the equivalence of results requires confirmation by standard or regulatory methods.

CDL operates with the reference methods published as ISO or ASTM documents. However, the CDL has to verify the characteristics of the method by 5.4.2 [2] "... The laboratory shall confirm that standard methods can be operated correctly before testing or calibration." Checking is necessary in case of significant changes, for instance when the device was previously replaced with a similar new one, the equipment was displaced, etc.

In laboratory medicine, most measurements and tests are performed using serial methods approved by the manufacturer, which, however, must be verified by the end-operate. In [12] it is stated that "research methods used without changes are subject to independent verification of CDL before their introduction". This also applies to situations where the software in the device has been updated or when quality control has shown that the characteristics of the method change over time.

At the end of the initial stage of developing the method, the CDL should develop a detailed document on the measurement methodology. There are two main approaches to method verification: interlaboratory comparison testing and validation in one CDL. Whatever the approach, the responsibility for ensuring that the method conforms to the task and for further operate on obtaining additional data to supplement the existing validation results rests with the CDL

After receiving a specific task from the customer, CDL must first establish analytical requirements, determine what characteristics of the method should have to perform the task (Fig. 1).

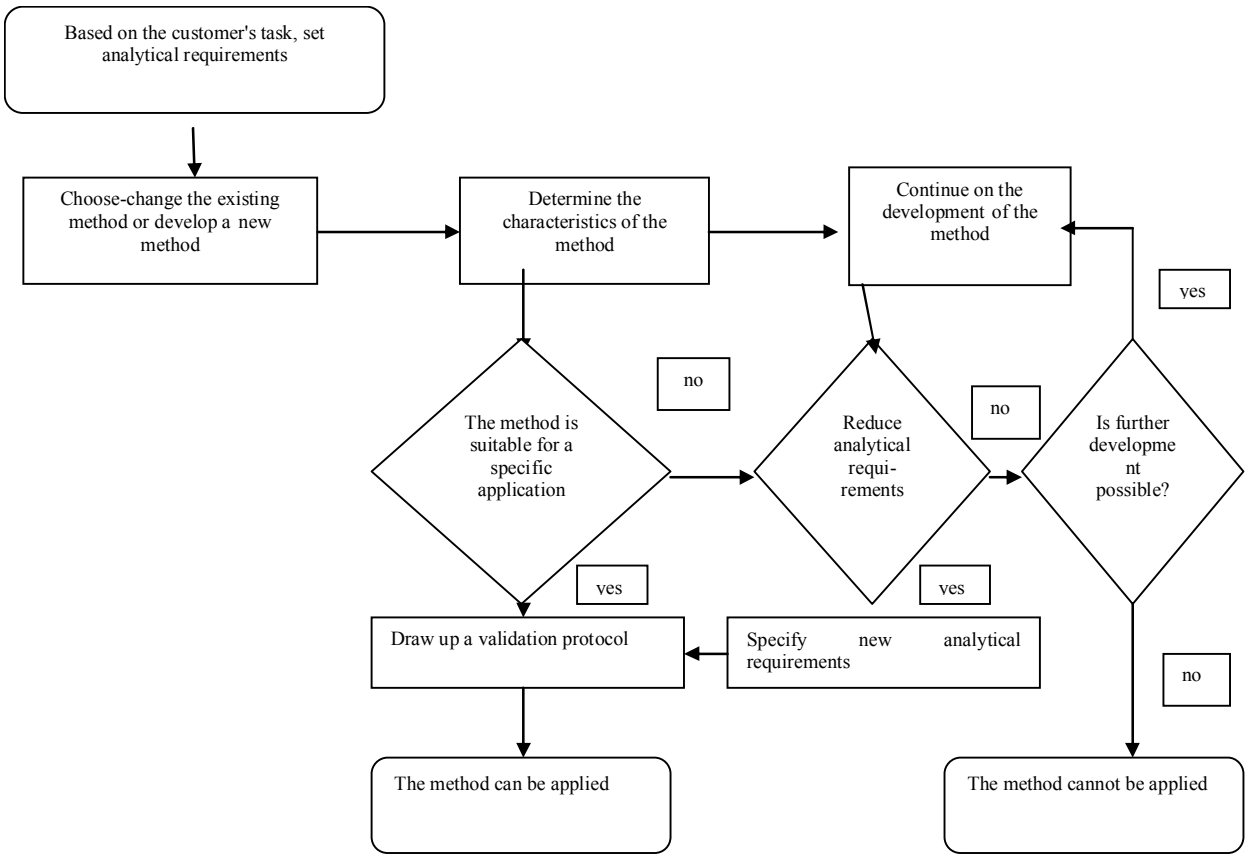

Fig.1. Method validation process: from the task set by the customer to the decision of the laboratory on whether it is possible to satisfy the customer's request using a particular method

Validation of the method involves the stage at which the characteristics are evaluated and compared with the analytical requirements. Regardless of the characteristics of the method, its suitability for users is determined by what characteristics a particular analyst receives on existing instruments. Based on these requirements, CDL selects the appropriate existing method or, if necessary, develops or modifies the method. Note that some guidelines may require the operation of a particular method. The validation process ends with a conclusion on the compliance/non-compliance with the requirements of the analytics. If the analytical requirements are not met, it is necessary to continue operating on the method. This process of development and evaluation continues until the method is recognized as meeting the established requirements.

Confirmation of method validation using specially organized interlaboratory comparisons often is so-called "joint research" or "cooperative research". Usually is sufficient for the consumer to check the published data on the characteristics and/or the set of characteristics of the method for their operation. Therefore, this approach reduces labor costs in CDL, which makes go this method [13].

In CDL practice there are cases when it is necessary to apply a certain method, but the corresponding standard is absent. If a method is designed to operate within one CDL, for example, due to a lack of general interest in that method or because other CDLs are competitors, this is justified by validation in the CDL. The latter determine which features should be investigated to test the method, and in some situations and how long examine each one. If the scope of analytical operation is clearly defined and the task is performed by the same organization or its department, the general guidelines for the study of the validation scope may be issued. Table 1 presents an example of analysis.

Evaluation of the characteristics of the method may be limited by various factors. This is recognized in [2]: "Validation is a compromise between cost, potential risk, and technical capabilities" [14]. Within certain limits, CDL should perform as much as possible, taking into account customer requirements and regulations, gained experience, available technical means, and ensure metrological compatibility with other similar methods used by the laboratory.

Some properties can be identified during the development of the method or its implementation. It is often possible to obtain certain data on several characteristics from a specific set of research results; then rational planning permits not determining resources to obtain the necessary information.

Table 1

For four types of analytical tasks, the amount of validation

Major characteristics	Task type (analytical)			
	Manifestation	Impurities, their quantitative determination	Compliance (verification) for the maximum content of impurities	The main component, its quantification
Selectivity	x	x	x	x
Detection (limit)			x	
Quantitative definition (limit)		x		
Working range (linearity)		x		x
Correctness (shift)		x		x
Convergence and intermediate precision.		x		x

Note. The 'x' icon indicates the characteristics that are determined during validation

The validation of methods is fulfilled regularly. The principle of regularity is involved also in episodic studies. The obtained results must be of the required level of reliability. Achieving a balance between time and money, on the one hand, and need, on the other, is quite difficult. Sometimes, it is advisable to perform certain analytical issues in another CDL that permanently fulfills it. For example, it was compared the quality and probability of determining the components in biological fluids by cyanide hemoglobin with the help of an automatic hematology system ADVIA 60 CT and UNICO 2800UV/VIS spectrophotometer. Statistical indicators were applied in processing the results. Validation was defined by the linearity of the method, convergence, reproducibility, accuracy, and uncertainty of characteristics.

3.1. Procedure for testing the method of determining hemoglobin

The scenario of the check is developed: technical features are established; the parameters to be evaluated are analyzed. Given the technical characteristics of the automatic hematology system and spectrophotometer, the detection limit of this technique is limited only by the characteristics of the test sets and is equal to 30.0 g/l. The factor of variation of the definition is lesser than 2%.

An inspection plan and inspection protocol are being developed. They identify the personnel participating in the inspection procedure by the qualification. Information and documentary confirmation of the proper operation of the used devices are being provided. Then the sequence of experiments, their number, form, and type of their provision, as well as the choice of acceptable for evaluation statistical methods of processing the results of measurements are being proposed. The experiment is planned to take into account the reasonable use of the results to evaluate several parameters. Given that the amount of data required may vary, it is recommended that at least three measurements be made for each batch.

Before conducting a study of interlaboratory convergence and reproducibility, we have analyzed the possible causes of lowering the accuracy in determining the hemoglobin and found that the main source of loss while operation seems to be the CDL operator. The ambient temperature factor does not take into account during the inspection procedure since the temperature was maintained permanently by the air conditioner.

To verify the influence of the factor "operator" on the convergence and reproducibility of the results in CDL, two laboratory operators of the same qualification performed three measurements on a standard sample of hemoglobin Cst = 118.0 g/l. The measurement results are given in Table 2 [15].

To assess the convergence of measurements performed in CDL, variances (H0: s 2.1 = s 2.2) were checked using Fisher's test. The sample value was calculated when operate on the automatic hematological system ADVIA 60 CT: F0.975 = 1.29 / 1.0 = 1.29; and when operate on a spectrophotometer UNICO 2800UV / VIS: F0.975 = 0.6561 / 0.64 = 1.012. The calculated values of this criterion are substantially smaller than the critical value of Fisher's criterion F0.975 (n1 = 9, n2 = 9) = 4026. It allows us to conclude that the variances are homogeneous and the samples belong to one general population; this indicates the absence of gross errors in the operation of the devices and statistically significant differences in the measurements of both operators.

Table 2 demonstrates the assessed results of the convergence and reproducibility variances while operating on two devices, as well as the limits of convergence (r 2.77 = ×σ) and reproducibility (R 2.77 S = ×R).

Verification of the method was performed on standard samples in terms of intra-laboratory convergence and reproducibility. Each series of measurements was checked for homogeneity. For a standard hemoglobin sample Cst = 118.0 g/l, one CDL-operator has performed 4 series of observations at different operating times of the automatic hematology system ADVIA 60 CT and the UNICO 2800UV/VIS spectro-

photometer. The systematic error was defined and its significance was checked in comparison with the random error according to the Student's eligibility criterion. Estimation of equality of average values employing this criterion showed statistical insignificance of differences in results of the measurements received at a different time of operation of both devices. It proves the correctness of the method and the belonging of the samples to one general population.

Table 2

Estimation of convergence of measurements at the check of influence of the factor "operator - laboratory assistant"

J	Haematological system ADVIA 60 CT		Spectrophotometer UNICO 2800UV/VIS	
	Operator 1	Operator 2	Operator 1	Operator 2
1.	119.0	117.0	118.0	120.0
2.	116.0	119.0	119.0	120.0
3.	117.0	117.0	118.0	119.0
4.	118.0	119.0	117.0	120.0
5.	117.0	118.0	118.0	120.0
6.	119.0	116.0	119.0	120.0
7.	119.0	120.0	118.0	118.0
8.	119.0	117.0	119.0	119.0
9.	118.0	118.0	120.0	121.0
10.	118.0	118.0	119.0	119.0
$\bar{X}_j$	118.0	117.9	118.5	119.6
S_j	1.0	1.14	0.81	0.8
$\bar{\bar{X}}$	117.95		119.05	
σ_r	1.07		0.803	
S_B	0.05		0.55	
S_R	0.733		0.84	
r	2.964		2.225	
R	2.031		2.324	
U_p	6.37		6.31	

According to the measurement results, the expanded uncertainty Up was computed as a metrological assessment of suitability. It has proved that in the range of 117.95 ± 6.37 - /l and 118 ± 6.31 g/l, respectively, the obtained hemoglobin values can be considered as accurate and reliable.

When assessing the linearity of the method of determining the concentration of hemoglobin, 5 series of measurements of concentrations of 115.0, 117.0, 118.0, 119.0, 120.0 g/l have been performed. At least 3 measurements were performed in each series. Verification of the linearity of the method showed that according to the specified acceptance criterion (R2 = 9689) the linear dependence of the measured value on the calibration concentrations was noticed.

Taking into account the high-mentioned data, a comparative assessment of the obtained results and validation characteristics was performed. It was proved that the results of determining the concentration of hemoglobin and biological measurements fulfilled with help of ADVIA 60 CT can be compared with similar results obtained with a spectrophotometer Unico 2800UV/VIS. Thus, the measurements on these two devices are comparable, accurate, and reliable.

During the validation studying of the method "Determination of hemoglobin concentration" while operations on the system ADVIA 60 CT and spectrophotometer UNICO 2800UV/VIS, the particular features, approaches, and requirements for assessing the suitability of laboratory methods and key points of uncertainty were defined. The results of determining the concentration of hemoglobin on ADVIA 60 CT and spectrophotometer UNICO 2800UV/VIS confirmed the accuracy and reliability of the obtained data. Evaluation of intra-laboratory convergence, reproducibility, and correctness of the method characterizes the qualitative operation of the device and the absence of statistically significant deviations in the study.

3.2 Automation of planning, registration, and computing during verification of methods within the requirements of the standard

Let's consider the option of solving the current technical problem that is the automation of planning, registration, and computing during the verification of methods within the requirements of the standard [2]. The effectiveness of the proposed solution is shown in the example of implementation in the laboratory practice of the testing laboratory of SE "Agmintest", which allowed to abandon paper media for registration of verification data and significantly reduce the cost of CDL requirements for verification of methods.

To assess the correctness of the operator of standard methods, 2 criteria were chosen: estimation of the reproducibility (interlaboratory component) and the repeatability (convergence), and, accordingly, comparison of the obtained characteristics with the requirements of the method, i.e. the verification of repeatability and reproducibility. The CDL may assume that one or both criteria can be applied to assess the correctness of the operator of the standard method, following, f. i., the Eurachem recommendations. While assessing reproducibility and repeatability, the standard deviation and the relative standard deviation of results have been accepted which is a common practice.

AIS "Laboratory" generates a log of intermediate results, including the results of studies of parallel samples. So, data for the recurrence calculation are already in the

system, and there is no necessity to introduce them. While reproducibility assessing the system is based on the standard sample results and the results obtained during tests aiming at the implementing of intra-laboratory quality assurance techniques. That is, reproducibility and repeatability checks do not require the contractor to enter any new information about the test results.

To average and estimate the mean repeatability, the system attracts the RMS-dependence given in [4]. Since the data required for verification are entered while recording the test results, the report becomes simpler. The contractor selects the methodology and the "Audit Reports" section, where he can view existing reports and create new ones. To generate a new verification report, the contractor selects the period during which the system should take data from test logs and test results of the customer's samples:

– To assess repeatability or test results of standard or stored samples only;

– To assess repeatability and reproducibility.

The system automatically accepts all laboratory test results according to this method over time and generates a report ready for approval to verify the considered method [16]. After that, the CDL-head is reminded through the system menu of the need to approve the report. The system automatically identifies the verification report; uses the results of tests with dates and identification of relevant samples of CDL-customers; evaluates the repeatability of each sample, the overall repeatability; compares with the repeatability limit for the method and concludes on the correctness of operation, based on the assessment of repeatability. The results of the latter can be considered annually as containing the reproducibility due to changes in a row of impacts that continued throughout the year affect the results.

4. Conclusions

1. The analytical laboratory process is considered as a unique operating concept provided the specifying the errors, reducing their number and thus minimizing the risks, both at the initial stage, which includes patient identification and selection of tests and at the final stage, while processing and interpreting data.

2. The validation of the method is a process of establishing analytical requirements and confirming compliance with the capabilities of the method. An integral part of it is the evaluation of the characteristics of the method.

3. An important point seems to be the assessment of the method's suitability since the validation of the method was previously limited to the evaluation of its characteristics.

5. Acknowledgment

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6. Conflict of Interests

The authors state that there are no financial or other conflicts regarding operation.

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