

# VALIDATION AND VERIFICATION OF ANALYTICAL METHODS IN CLINICAL LABORATORIES

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## CONTENT

1.	Aim and purpose of recommendation.....	2
2.	The terms “validation” and “verification” (ISO 9000: 2000, Eurachem Guide. The Fitness for Purpose of Analytical Methods 1998) .....	2
3.	Why is validation/verification necessary? .....	2
4.	Who performs validations?.....	2
5.	When is validation/verification performed? .....	3
6.	Extent and intensity of validation and verification .....	4
6.1.	Verification of qualitative tests performed with the use of IVD products with CE marking .....	4
6.2.	Verification of quantitative tests performed with the use of IVD MD products with CE marking.....	4
6.3.	Validation plan of measurement methods of other quantitative tests.....	5
7.	General validation and verification procedure.....	5
8.	List of problems closely related to validation/verification.....	6
9.	Experimental and statistical method of validation and verification .....	6
9.1.	Repeatability, reproducibility.....	6
9.2.	Bias methods.....	7
9.3.	Comparability of methods.....	8
9.4.	Linearity and working interval of measurement .....	8
9.5.	Limit of detection and limit of quantification .....	10
9.6.	Recovery, analytical specificity and interference.....	10
10.	Validation of the measurement quality control programmes .....	12
11.	Summary of validation and verification principles.....	12
12.	Evidence of validation methods used before this publication .....	12
13.	Terminology .....	13
14.	References .....	14

## 1. Aim and purpose of recommendation

The subject matter of this recommendation is validation and verification of measurement methods and measuring systems in clinical laboratories.

The main aim of validation is to assess analytical and performance characteristics of methods and to make sure that the requested level of these characteristics was achieved. In laboratory medicine it means that results of measurements become effective diagnostic, therapeutic and prophylactic tools.

Validation of methods in clinical laboratories is required by quality management standards (ISO 17025, ISO 15189).

Results of validation and verification processes form:

- the basis for setting up quality management programmes (Eurachem Guide. The Fitness for Purpose of Analytical Methods: 1998, ISO/FDIS 15198: 2004);
- the basis for assessment of measurement uncertainties and for demonstration of traceability and comparability of measurement results.

## 2. The terms “validation” and “verification” (ISO 9000: 2000, Eurachem Guide. The Fitness for Purpose of Analytical Methods 1998)

Validation is confirmation that *the requirements for specifically intended use or specific applications were met* through objective evidence.

Verification means confirmation that the analytical characteristics data provided by a manufacturer, a laboratory or a reference institution were reached through objective evidence in the given laboratory with the use of a specific measuring system.

Validation confirms that the measurement method/measuring system/IVD MD product (in vitro diagnostic - medical device) is capable of meeting the requirements set on it. In other words, it confirms that the level of measurement is sufficient, the measurement procedures are correct and the calibration was properly done.

Verification means that the measurement method/measuring system/IVD MD product is fully functional in a specific laboratory. In this recommendation, the term "verification" will only be used in relation to the products marketed in accordance with the principles of the Directive IVD 98/79 EC. In other cases, the term "validation" will be used.

Objective data mean the results of planned laboratory experiments on the one hand, and the data from manufacturing documents, research laboratories, and certification procedures on the other hand.

Analytical characteristics that form the subject matter of validation/verification form the base of **validation/verification plan**.

## 3. Why is validation/verification necessary?

Results of analytical measurements have an extraordinarily great impact on practice. In clinical laboratory, they can definitely, and sometimes even fatally, influence health, quality of life, and even the patient's life.

It is the professional duty of analytical chemists to carry out measurements of sufficient quality.

Validation provides absolutely necessary data about uncertainty (confidence interval) of the measurement results.

Accreditation, which means official confirmation of a laboratory's competence, requires the use of properly validated and verified measurements.

## 4. Who performs validations?

### *Manufacturers of diagnostics*

According to the Directive IVD 98/79 EC, only validated measuring systems can be introduced to the European Union market. The procedure and extent of validation of IVD MD is specified by a standard (EN 13612: 2002).

Manufacturers who market their products in the USA must also validate their products within the extent required by the document "Guidance for Industry: Bioanalytical Method Validation", elaborated by the following institutions:

- US Department of Health and Human Services,
- Food and Drug Administration,
- Center for Drug Assessment.

This document which was issued in 2001 is available on <http://www.fda.gov/cder/guidance/index.htm>

#### ***Professional organizations of analytical chemists***

The aim of AOAC (the Association of Official Analytical Chemists) is to contribute to the worldwide credibility of analytical results (i.e. of analytical chemistry and analytical chemists as well). This association has developed a number of validation, verification and certification programmes and cooperates with such institutions like FDA, thus targeting directly laboratory medicine as well. Web site: [www.aoac.org](http://www.aoac.org)

LGC- Laboratory of the Government Chemists ([www.lgc.co.uk](http://www.lgc.co.uk))

Among others, it also operates VAM programme (Validated Analytical Measurement) available on <http://www.vam.org.uk>

#### ***Laboratories***

However, final responsibility for adequate validation of measurements is borne by the laboratory itself. If validation data come from the manufacturers' documents and are supported by experiments in laboratories of manufacturers and professional organizations and in calibration/reference laboratories, then the laboratory must continuously complement and verify such data. Following means are available to reach this aim:

- continuous education of the staff;
- regular quality controls (intra- and inter-laboratory comparison);
- elaboration and revision of SOPs and their validation during which the main emphasis is placed on assessment of results of inter-laboratory comparisons and studies;
- continuous observation of documents and information provided by reference institutions and organizations, such as JCTML (Join Committee for Traceability in Laboratory Medicine), LGC, and AOAC;
- observance of standard operation procedures meeting the requirements of the Directive IVD 98/79 EC.

***The trend of modern analytical measurement is clear: Validated kits and measuring systems should be used and laboratories should concentrate mainly on assessments of measurements uncertainty, quality control, education, continuous observation of information, and implementation of new findings in practical laboratory activity.***

## **5. When is validation/verification performed?**

- When a new method is being introduced;
- During the purchase and before application of a new analytical measuring system in the laboratory;
- When a new (different) diagnostic kit is being introduced;
- When existing methods are being enlarged by another purpose (such as enlargement of measurement by another kind of biological material);
- When a perennial problem is shown by quality control;
- When a method of in-house type has been adopted from another laboratory or publication;
- One year later (revalidation/reverification).

## 6. Extent and intensity of validation and verification

Extent and intensity of validation must always correspond with the need to get sufficient amount of data enabling to decide whether the method is really suitable for the intended purpose (ISO 17025, ISO 15189). Validation plans depend on the character of the validated method. The validation of a qualitative method requires much lesser effort, than the validation of a quantitative method. The methods validated by the manufacturer - IVD MD products with CE marking require lower extent of validation than the in-house methods either developed by the laboratory itself or modified by the laboratory, or those taken over from another laboratory.

Extent and intensity of validation are described in and determined by the validation plan.

From the point of view of validation and its required extent, the methods can be divided in four groups:

- (1) Qualitative tests performed by means of IVD products with CE conformity marking;
- (2) Quantitative tests performed by means of IVD products with CE conformity marking;
- (3) Other quantitative tests performed by means of IVD products with CE conformity marking but using a modified procedure;
- (4) In-house type quantitative tests.

Validation and verification in the above mentioned groups of methods differ diametrically. Our recommendation is based on LGC-VAM published in 2003 (VAM Bulletin, 28, 2003, 17-21). This material, developed by metrologists with high erudition and international authority, distinguishes the following levels of extent and intensity of validation and verification:

### *Validation of a validated method*

Validations of products that comply with the requirements of the Directive IVD 99/79 EC and provided with CE marking belong to this group. The laboratory will verify only the basic prevalidated parameters (see below).

### *Validation of modifications of an originally validated method*

Use of a kit/IVD MD system, the procedure of which was modified in some way, belongs to this group. Such a modified kit should undergo "cross" validation. It is performed (Guidance for Industry-Bioanalytical methods validation <http://www.fda.gov.com>) by comparison of results of validation of both, the original and the modified kit. The aim is to document that modification has not impaired the conformity of properties with the requirements specified for the given use.

### *Validation of a new method*

In that case, an in-house type method is concerned and it must be validated in full extent.

#### **6.1. Verification of qualitative tests performed with the use of IVD products with CE marking**

In this group, the only requirement is to observe strictly the manufacturers' operational procedures (CLIA 88 – Final CLIA Rules Part V, available on <http://www.wesgard.com> and in the book by J.O. Westgard: Basic Method Validation).

This type of methods should have the program of internal quality control. According to the Directive 98/79 EC, the control materials should be recommended, supplied and described by IVD manufacturer in accordance with ISO 15198: 2004.

#### **6.2. Verification of quantitative tests performed with the use of IVD MD products with CE marking**

Most laboratory tests belong to this category. CE marking in IVD products means confirmation of their properties conformity with the required ones, i.e. confirmation that they were found to comply with the requirements of the European Directive 98/79 EC before their marketing. IVD manufacturer's system of quality control thus corresponds to ISO 13485 standard and their products have been validated by the procedures required by EN 13612: 2002 standard. The fact that the laboratory uses products complying with the Directive 98/79 EC results in its following duties which allow to demand manufacturer's responsibility for an IVD MD product:

- - the product must be used in a strict conformity with its intended use declared by the manufacturer;
- - the laboratory must observe the procedure specified by the manufacturer (it must not modify this procedure in any case and in any way);
- - the laboratory must interpret the results of these tests in accordance with the international recommendations (if available);
- - instead of extensive validation, the laboratory performs basic verification of such products' properties, using a simple verification plan.

The aim of laboratory verification of IVD methods is to confirm that the achieved values of analytical and performance characteristics conform to the values declared by the manufacturer and that they can be achieved in the given laboratory under common conditions of routine operation.

Thus, it is not the IVD product itself that is the subject matter of verification but the ability to implement the measurement procedure in a specific laboratory in a given time and space.

A reliably estimated value of a measurement uncertainty should be the decisive result of verification.

Verification plan of IVD MD products (minimum):

- A) Precision (repeatability, reproducibility)
- B) Recovery as quantification of bias value

*These characteristics are necessary to assess the measurement uncertainty. If needed, the laboratory can verify other analytical characteristics as well.*

***If the IVD MD analytical system is modified in any way, we must perform the "cross" verification by means of which precision and recovery (and then the measurement uncertainty) of the measuring system before and after modification is assessed. We must prove that the modification did not cause any significant deterioration of these analytical characteristics. In addition, analytical characteristics of measurement, results of interlaboratory comparisons and external quality control must be documented to prove that the modification did not cause a breach of conformity.***

### 6.3. Validation plan of measurement methods of other quantitative tests

Methods developed by a laboratory or methods adopted from another laboratory (in house) belong to this category. In such a case, much more extensive and comprehensive validation plan must be used.

- A) Precision (repeatability, reproducibility)
- B) Recovery – bias
- C) Linearity/Measuring interval
- D) Limit of detection/quantification
- E) Interference
- F) Comparison with another method

## 7. General validation and verification procedure

- Setting of requirements that shall be reached for a specific purpose;
- (In IVD - MD products, these requirements form a part of operational documents supplied by the manufacturer and the laboratory verifies their validity only.)
- Determination of validation/verification extent, elaboration of a plan;
- Performance of appropriate experiments
- Assessment of results
- Adoption of measures to satisfy the specified requirements, if necessary;
- Elaboration of validation/verification documents.

## 8. List of problems closely related to validation/verification

- Purpose of measurement;
- Definition of the analyte;
- Sampling; sample transport and handling;
- Summary of minimal requirements of the measuring device quality
- Sequence of measurement standards;
- Calibration of measurement;
- Precision;
- Bias and recovery;
- Checking of the used calculations validity;
- Sensitivity, linearity, measuring interval, limit of detection/quantification;
- Robustness;
- Uncertainty of measurement;
- Determination of quality control limits;
- Recording of validation studies results;
- Validation/verification documents;
- Statement on the ability of the method to comply with the specified requirements;
- Comprehensive documentation of the quality control.

*All above given items must form an integrated, continuously solved and monitored complex in the laboratory. The solution of partial problems without a link to the others is irrelevant.*

## 9. Experimental and statistical method of validation and verification

### 9.1. Repeatability, reproducibility

#### Aim

Quantification of the random error of measurement

Number of samples, replicates; concentrations range

- 20 measurements of two samples of two different concentrations (one sample within the reference limit, the other one above the upper reference or decision limit, or below the lower limit of the reference interval)
- Both patients' samples and control/reference materials can be used alternatively. To determine repeatability and reproducibility, the same samples can be used.
- The value of the standard deviation of repeatability (in a series of 20 measurements) and reproducibility (each of both samples is measured in a singlet for 20 consecutive days) is determined.

*Eurachem Guide (The Fitness for Purpose 1998) mentions the possibility to calculate repeatability and reproducibility using 10 measurements.*

Statistical assessment

- Mean value
- Standard deviations of repeatability and reproducibility
- Variation coefficients of repeatability and reproducibility

## 9.2. Bias methods

### Aim

The estimation of systematic error of measurement by means of analysis of either reference or suitable control material. To perform such an estimation, the random error of measurement must be minimized by means of sufficient number of repeated measurements.

Bias value:

- records the traceability of measurements in the laboratory;
- quantifies the systematic part of the combined uncertainty of measurement results

### Samples

Matrix certified reference materials that have the values of analytes obtained by reference methods of measurement are the ideal samples for determination of the measurement bias. Such samples are not available for a routine laboratory. In practice, a laboratory compares its results with the results of other laboratories using the same control materials. Such a comparison can be performed by means of control materials used previously in programmes of external quality assessment (NCCLS EP9-A2, NCCLS EP15-A, Eurachem Guide. The Fitness for Purpose of Analytical Methods, 1998, FDIS VIM 3ed., 2004).

Such materials are measured under repeatability conditions. The bias value reached under the repeatability conditions is suitable for the assessment of uncertainty of measurement.

### Replicates, concentrations

10 measurements under repeatability conditions (in one series), 2 reference (control materials - CM) of two different concentrations. One concentration of CM within the reference interval and one concentration of CM above its upper limit or below its lower limit.

### Reference values

According to the measurement traceability status and regarding the possible influencing of bias results by matrix effects, the following values are used:

- a) Reference values acquired by means of a reference method in an external reference/calibration/expert laboratory - RMP or AV.

Use of these values is subject to the condition that the difference between them and the mean results of participants does not exceed the amount of extended uncertainty RMP/AV ( $k = 2$ ).

Thus the relation:  $|RMP (AV) - ALTM| \leq U_{ref} (k = 2)$  must be true

- b) The mean of all results of participants of interlaboratory comparisons (after the exclusion of outliers), i.e. results of ALTM type.

These values are used if the above given relation between RMP/AV and ALTM is not true.

- c) The average of results of participants who use the same method of measurement.

These values are used in cases depending on the used measurement method/measuring system, i.e. values of ConV type. In such a case, the bias can only be assessed if the given group consists of 10 participants at least.

Procedure and calculation (Eurachem Guide. The Fitness for Purpose of Analytical Methods, Harmonised guidelines for the recovery information in analytical measurement):

Ten sample replicates are measured ( $x_1$  to  $x_{10}$ ) under repeatability conditions.

We get a set of 10 results  $x_i$  ( $x_1$  to  $x_{10}$ ).

Arithmetic mean AM is calculated using the formula:  $AM = (\sum x_i) / 10$

Repeatability value SD and CV (%) is calculated.

Bias value is calculated in form of recovery  $R_m$ .

$$R_m = AM / \mu$$

or

$$R_m (\%) = (AM / \mu) \cdot 100$$

where  $\mu$  is the reference value (one of the above given possible values).

### 9.3. Comparability of methods

#### Aim

Assessment of bias, type of systematic error and correlation by comparing a validated method with a comparative one.

Reference measurement method is an ideal comparative method. In routine practice, it is not usually available. Therefore, a new routine method/procedure/analytical system is compared with another routine method/procedure/system which was validated previously. If POCT method/system is validated, we use routine laboratory methods as comparative ones.

#### IVD-MD products

The manufacturer is obliged to provide the laboratory with data about traceability and comparability of its method (ISO 17511, ISO 18153). The manufacturer must prove the comparability by a graph and numerical assessment of regression curve or by means of Bland-Altman diagram. This duty is stipulated in standards (EN 375, ISO 18112-1).

#### Samples

For in-house methods, the use of at least 40 samples of native biological materials from patients is recommended. The samples must be chosen carefully to cover uniformly the whole working measuring interval (J.O. Westgard: Basic Method Validation 2003). Careful choice of limits from the point of view of suitable concentrations is considered much more essential than increase of their number.

#### Replicates

Measurement of individual samples in duplicates is recommended. The experiment may be performed in one day, however, 5 days by 8 samples each are recommended.

#### Results recording

It is recommended to record the measured data in a table (such as Excel). The results of comparative methods are always recorded in the "x" column and results of validated method in the "y" column.

#### Graphical data recording

- Bland-Altman diagram, where the "x" axis shows the means of both methods and the "y" axis shows differences of both methods (in % preferably).
- Regression graph, where the "x" axis shows localized value of comparative method.

Use of Passing-Bablok nonparametrical regression is preferable to linear regression (OLR).

#### Assessment of systematic difference between the validated method and the comparative one

From the equation  $y = q + k \cdot x$  the value of bias (b) is determined according to:

$$b = y - x$$

This procedure is only recommended when correlation coefficient  $r \geq 0.975$  is reached. It is Pearson's correlation coefficient where the values of the slope (k) and section (q) can be considered correct.

It can be concluded from the graphical record of Bland-Altman diagram, the graphical record of regression analysis, and from the value of the regression analysis slope whether the proportional part of systematic measurement error is present or not.

It can be concluded from the graphical record of Bland-Altman diagram, the graphical record of regression analysis, and from the value of the regression analysis section whether the constant part of systematic error is present or not.

### 9.4. Linearity and working interval of measurement

#### Aim

Checking the validity of linearity interval/working interval of measurement under the conditions of a specific laboratory. This interval defines the range within which the validity of precision and bias values declared by the manufacturer and checked by validation experiments can be safely expected.



### IVD-MD systems

According to IVDD (98/79 EC) and in accordance with the standards EN 375 and ISO 18112-1, the manufacturer is obliged to provide the laboratory with validated data about linearity and working interval of measurement. In IVD-CE products intended for immunoanalytical measurements, working interval is characterized by data obtained by means of a dilution experiment and quantified by recovery values (R %).

### Material for linearity validation of in house methods

Commercially available kits of reference materials intended directly for this purpose can be successfully used. Such kits can be used within the appropriate programmes of external quality assessment (such as "Linearity" programme, SEKK, in the Czech Republic).

In case of analytes with no such material available, measurement by means of a kit of human sera is recommended for linearity checking. If verification of the working interval of measurement does not provide linear relation between the analytical signal and concentration, dilution experiment is used.

### Preparation of the set of samples for linearity validation (J.O.Westgard, Basic Method Validation, 2003)

A pool of patients' samples (H) of extremely high analyte concentration is prepared. The concentration should be close to the upper limit of linearity/working interval declared by the manufacturer; however, it must not exceed it.

A pool of patients' samples (L) of extremely low analyte concentration is prepared. The concentration should be close to the limit of quantification. If a "zero" calibrator is available, it is used as the "L" sample.

By mixing the both pools, other three samples are prepared (see the table).

Table of samples for validation of linearity/working interval:

Sample 1	L
Sample 2	3L + 1H
Sample 3	2L + 2H
Sample 4	1L + 3H
Sample 5	H

*Precision and trueness of volumes measurement are the critical points of this experiment.*

### Preparation of samples for dilution experiment

Five (or more) samples with decreasing analyte concentrations are prepared from biological material whose concentration is close to the upper limit of the working interval. By means of choosing more samples with suitable initial concentrations, the whole working interval of measurement can be covered (or its clinically most important part at least).

*Example (measurement of TSH concentration in blood serum):*

Dilution	Measured value - mean	Theoretical value	R (%)
1	19.2	20	96
3	7.1	6.7	106
5	3.88	4.0	97
9	2.25	2.2	102
17	1.27	1.18	108

### Replicates

Each sample is measured in triplicate. The sequence of individual samples should be randomized.

### Assessment

In linearity validation, the dependence of the means of measured values (y axis) on theoretically known values (x axis) is assessed. Linear regression is used for assessment.

In case of validation by means of a dilution experiment, recoveries of individual samples (R %) are assessed according to the equation:

$$R \% = (\text{result of measurement/theoretical value}) \cdot 100$$

### Procedure recording

- Graph of dependence of measured values (y axis) on theoretical values (x axis);
- Slope, section and correlation coefficient
- Percentage of recovery (R %)

## 9.5. Limit of detection and limit of quantification

### Material

Sample blank or "zero" calibrator.

### IVD-MD systems

The manufacturer provides the user with the value of the detection limit. The value of quantification limit is not always provided. If the limits of quantification in IVD-MD products are available, the (confusing) term "functional sensitivity" is often used.

### Procedure of determination of the limit of detection (LoD)

- Ten blank samples are measured in a series.
- The value  $3 \text{ SD}_{\text{blank}}$  is calculated.
- LoD value is calculated as concentration corresponding to  $3 \cdot \text{SD}_{\text{blank}}$  (by means of routinely used calibration).

### Procedure of determination of the limit of quantification (LoQ)

- The limit of quantification is calculated according to the equation
$$LoQ = 3 \cdot LoD$$
- Reproducibility is determined in several samples (ten are recommended) by means of measurement in ten different days. The lowest reached value of sample concentration in which better than required reproducibility is reached, will be used as the limit of quantification. In clinical laboratories, the target reproducibility should be  $CV = 20 \%$ .

*The limit of detection belongs to key analytical characteristics of qualitative methods using nominal and/or ordinal measuring scales.*

*The limit of quantification belongs to key analytical characteristics of quantitative methods of measurement. It is sometimes wrongly named "functional sensitivity".*

## 9.6. Recovery, analytical specificity and interference

### Aim

Recovery of measurement obtained by adding the measured analyte to a sample of measured material quantifies the bias of measurement (see above).

The values of measurement recovery obtained after adding an interferent to a sample of measured biological material provide information about analytical specificity of the validated method by means of assessment of presence or absence of a significant proportional systematic error of measurement. In separation methods, recovery of measurement forms the basic information about efficiency of the compound isolation from the sample matrix. In these cases, recovery is determined by adding a suitable amount of analyte in a biological material sample.

### **Procedure and calculation of recovery**

The simplest procedure is to measure (certified) reference material or control material validated in EQA programme. Ten measurements are recommended under repeatability conditions and two reference (control) materials on two different clinically significant levels. The procedure and assessment of recovery R was described above in the section about bias measurement.

If there is no commutable certified reference material available during the validation (which is quite common), addition of the analyte to the native patients' material is used instead of it. If the aim of the experiment is to assess the analytical specificity of measurement and the level of interference, then the method of the assessed interferent addition is used.

#### Number of patients' samples

In most cases, a few (approximately 5) samples suffice.

#### Analyte addition

It is recommended to add nonmatrix reference material, usually its water solution, by means of adding a small volume of it directly to the samples of biological material (such as patients' samples).

The concentration in patients' samples and the concentration of the added analyte must be chosen so that the resulting concentration in measured samples was:

- within the linearity interval/working interval;
- within the range of clinical decision limits, if possible.

#### Interferent addition

It is carried out according to the same principles as those specified at recovery determination. Instead of the analyte, an interferent is added. These are the basic tested interferents:

- haemoglobin (added in form of erythrocytes haemolysate);
- bilirubin (added in form of a standard solution);
- triacylglycerides (added in form of commercially available lipidic suspensions).

#### Volume of the added analyte - interferent

It must be as least as possible so as not to dilute the patients' samples matrix significantly (mixing of 9 parts of the patient's sample with one part of the added analyte sample is recommended). The base solution of the added analyte must be duly concentrated (tenfold addition in the example given above) and very high precision of volumes measurement must be ensured. Balances and volume-measuring instruments linked to reference must be used for substances weighing and volume measurement.

#### Preparation of samples for recovery measurement

For each patient's sample used for the experiment, a partial sample with the addition (sample A: 9 parts of the original sample + 1 part of the addition) and a partial sample with dilution (sample B: 9 parts of the original sample + 1 part of saline solution or zero calibrator) is prepared. Samples A and B are measured in duplicates.

#### Calculation

- Concentration of the addition in the patient's samples is calculated according to the equation:

$$A_r = \text{concentration of the addition} \cdot \text{volume of the addition} / (\text{volume of the addition} + \text{volume of the sample})$$

- Mean values of A and B samples duplicates are measured.
- Difference of A and B is calculated and the mean value of their duplicates  $D_r$  is determined.
- Recovery as ratio  $D_r/A_r$  is calculated.
- Percentage of recovery is calculated:  $R = (D_r/A_r) \cdot 100$  [%]

### **Interference**

In IVD-CE products, the basic interference data form a part of the manufacturer's working documents. Such basic data include information about haemoglobin, bilirubin, lipids and drugs interference, as well as data about cross reactions.

## Records

- The graph of dependence of theoretical concentration values on the measured values. Interferences occurrence can be detected well by the breach of linearity, i.e. by the change of the graph's slope which shows the presence of proportional systematic error caused by the interferent.
- Numerical values of recovery (R and R %).

## 10. Validation of the measurements' quality control programmes

According to the standard ISO/FDIS 15198: 2004 (Clinical Laboratory Medicine. In vitro diagnostic medical devices. Validation of user quality control procedures by the manufacturer), IVD-MD manufacturer is obliged to provide the user of the product - the analytical measuring system - with the following data required for implementation of the quality control:

- Specification of the type of errors that can be detected by the quality control programme;
- Specific recommendations of control materials that should be used;
- Recommendations of analytes concentrations that should be used;
- Recommended values of control limits;
- Specifications of the limitations of the quality control process.

Quality control must be considered a continuation of validation/verification procedures. The results of validation/verification experiments, especially precision, bias measurement, and criteria of suitability for intended use (control limits) are the data that the quality control programme is based on.

*The standard ISO/FDIS 15198: 2004 deals only with validation of programmes of internal quality control. Control materials for implementation of external assessment of quality are not a part of IVD MD directives.*

## 11. Summary of validation and verification principles

- Collect and record validation parameters and analytical characteristics (manufacturers' data about analytical characteristics of a method/measuring system).
- Collect and record the appropriate criteria (quantity of analytical characteristics specifying requirements for the analytical system to meet the intended use).
- Plan the verification/validation experiments.
- Before the experiments are started, acquaint sufficiently with the device and the method. Instruct the staff in details. Check the computing technique including statistical programmes.
- Carry out the experiments.
- If necessary, reassess the criteria and repeat the experiments.
- Summarize and assess the results in form of a validation report.
- Propose the procedure of quality management (IQC, EQA)
- Elaborate the appropriate SOPs.
- Determine the criteria and plans of revalidation.

## 12. Evidence of validation methods used before this recommendation issue

In methods used before the issue of this recommendation, validation can be proved by internal quality control data and records of external quality assessment and/or records of interlaboratory comparison programmes. Data of working documents of IVD MD manufacturers complying with the requirements of the Directive 98/79 EC are certainly considered to be the data proving validation of the method.

The laboratory must have complete primary data at its disposal from the measurements carried out during validation experiments and all the primary data of statistical assessments.

### 13. Terminology

*The following terminology is based on the electronically published text "Metrological Terminology in an Analytical Laboratory", SEKK 2003. The terms are defined in a short version sufficient for the given purpose. The text of the mentioned CD-ROM is based mainly on normative texts of ISO 3534-1, ISO 57287-1 and VIM (3rd edition).*

<b>Analytical interference</b>	The systematic error of measurement caused by the analytical interferent.
<b>Analytical interferent</b>	A part of the sample interfering with the measured value. It need not be a source of the measuring system signal but causes either increase or decrease of its indicated value.
<b>Blank sample</b>	A sample that does not contain the analyte of interest or has a concentration at least an order of magnitude less than the lowest level of interest.
<b>Error of measurement</b>	The difference of the quantity value obtained by measurement and the true value (reference value) of the measurand.
<b>Random error</b>	A part of an error of discrete and variable character. The difference of the quantity value obtained by measurement and the average that would ensue from an infinite number of replicated measurements of the same measurand carried out under repeatability conditions. It can neither be influenced nor corrected mathematically; it can be both of positive and negative value. It causes a measurement results variability which can be characterized by precision of measurement quantified in form of standard deviation or variation coefficient on the basis of statistical analysis of a series of independent measurements. The influence of a random error on the measurement result can be decreased by an increase of number of measurements.
<b>Systematic error</b>	The difference of average that would ensue from an infinite number of replicated measurements of the same measurand carried out under repeatability conditions and the true value of the measurand. It is such a part of error which remains constant in a series of test (measurement) results, or which changes in a predictable way.
<b>IVD MD (In Vitro Diagnostic Medical Devices)</b>	European Directive (IVDD) 98/79 EC defining the conditions and requirements necessary to market laboratory diagnostics and devices in EU countries (transferred in legislation of individual EU countries in form of a law on 1 <sup>st</sup> June 2000).
<b>Control material</b>	The material used for the purposes of an operational quality control both for internal quality control and external quality assessment. It undergoes the same measurement procedure (or its part) as analyzed samples aiming at effective monitoring of the analytical quality of measurement.
<b>Linearity of calibration relation ("linearity" in short)</b>	<p>The ability to provide results that are directly proportional to the concentration; the range of content, amount or concentration values in which the analytical signal is a linear function of content, amount or concentration values.</p> <p>Calibration is an operation that establishes the relation, obtained by the reference to one or more measurement standards that exists under specified conditions between the indications of a measuring system and the measurement result that would be obtained using the measuring system</p>
<b>Limit of detection</b>	The limit of detection of a specific analytical procedure is given by the lowest amount of an analyte in a sample that can be detected with stated probability, although perhaps not quantified as an exact value with desired uncertainty level.
<b>Limit of quantification (lower LoQ)</b>	The limit of quantification of a specific analytical method is the lowest amount of an analyte in a sample that can be quantitatively determined with stated acceptable precision and trueness with required uncertainty level.
<b>Measurement</b>	The set of activities aimed to determine the value of a quantity. The process of experimentally obtaining information about the magnitude of the quantity.
<b>Measurement method</b>	The generic description of a logical sequence of generally described

	operations - activities used in a measurement.
<b>Measuring system</b>	The complex set of all constituent parts enabling performance of individual specified measurements.
<b>Measurement procedure</b>	A detailed description of measurement activities according to one or more measurement principles and to a given measurement method.
<b>Trueness</b>	The closeness of agreement between the average that would ensue from an infinite number of quantity values obtained under specified measurement conditions and the true value of the measurand.
<b>Precision</b>	The closeness of agreement between quantity values (independent results of a test) obtained under specified conditions. These conditions include repeatability or reproducibility.
<b>Specificity (analytical)</b>	The ability of a measurement procedure to determine only that measurand which is to be measured. The lower the interference is, the higher is the specificity.
<b>Bias</b>	The difference of the mean quantity value obtained from a great number of measurements and the reference value (true value) of the measurand – systematic error.
<b>Recovery</b>	The ratio of the measurement result to the certified - reference value. The indicator of the constant systematic error of a measurement or a systematic error caused by the interferent or an incomplete extraction of the analyte from the matrix.
<b>Validation</b>	A confirmation through the examination of a given item and the provision of an objective evidence that it fulfils the requirements for a stated intended use.
<b>Verification</b>	A confirmation through the examination of a given item and the provision of an objective evidence that it fulfils the specified requirements.

## 14. References

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