

## 1. Foreword

This document has been drawn up by a working group appointed by DANAK's sectoral committee for diagnostic specialities, consisting of a representative from DANAK and members appointed by DSKB

## 2. Object and delimitation

Guidelines, made by DANAK, express DANAK's interpretation of relevant paragraphs in the accreditation criteria, made to ensure a harmonisation of treatment and assessment of laboratories, applicants and accredited laboratories and to harmonise the handling of Danish laboratories with laboratories in other countries.

The present guideline specifies DANAK's interpretation of the requirements in DS/EN ISO/IEC 17025:2000 and DS/EN ISO 15189:2003 for clinical biochemistry laboratories, concerning documentation of methods – especially their decisions in relation to necessary validation of performed analyse methods, and to assessment of the results of such a validation work.

Procedures, which cover nominal characteristics, are not included

The guideline is applicable to all clinical biochemistry laboratories, which have an accreditation or apply for an accreditation for testing or examination

## 3. Terms, definitions and references

References to ISO 17025 and 15189 are marked with [ ]. References to literature are marked with ( ).

ISO 17025 [5.1.5.1]: "Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled".

ISO 17025 [5.4.5.2]: "The laboratory shall validate non-standard methods, laboratory-designed / developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application. The laboratory shall record the results obtained, the procedure used for the validation, and a statement as to whether the method is fit for the intended use."(1).

ISO 15189 [5.5.2]: "The laboratory shall use only validated procedures for confirming that the examination procedures are suitable for the intended use. The validations shall be as extensive as are necessary to meet the needs in the given application or field of application. The laboratory shall record the results obtained and the procedure used for the validation ".The methods and procedures selected by the laboratory, shall be evaluated and found to give satisfactory results before being used for medical examination. (2)

ISO 15189 uses the term examination procedure. This document uses the term "analyse method" which is the frequent term used by Clinical Biochemical laboratories.

As part of the validation an estimate shall be carried out covering the total uncertainty related to the measure value, see pkt. 8.11. This uncertainty, matching the clinical requirements for using the test result, forms part of the basis on which the decision to use the method in the routine work is made.

#### 4. Scope

In ISO 17025 [5.4.5.2] standard methods and non-standard methods are mentioned. Standard methods are performed according to reputable specifications and are final validated. The only task for the laboratory is to verify that the method works locally. Within clinical biochemistry hardly ever any method is considered as validated. Often the used methods are scientifically published methods which are developed by the diagnostic manufacturer or by the laboratory or they may be developed by the laboratory itself. It can also be methods which are used outside their intended field of application or are modified. All such methods are to be validated.

The validation or part of it can be carried out by the manufacturer of the diagnostic service or by other laboratories. According to the IVD directive, since 2003 the manufacturer is obliged by law to carry out an extensive validation why the practical test done by the laboratory can be reduced accordingly. However the laboratory must ensure that the method can be properly performed in its own laboratory. Any how, the documentation corresponding to a total validation ought to be accessible to the laboratory.

#### 5. The content of a validation plan and - report

In ISO 17025 [5.4.5.2] is stated: "The validation shall be as extensive as is necessary to meet the needs of the given application or field of application. "

Therefore a validation plan (or protocol) has to be elaborated based on the purpose of the measurement and the practical tests have to be closed with a validation report including plan or protocol, worked out data and conclusions.

The protocol shall show the relevant quality objectives, see below. A validation plan or protocol will therefore include a detailed description of how relevant characteristics of the method (see below) are planned tested i.e. by getting documentation from the diagnostic manufacturer, by performing the examinations in own laboratory, scientific literature etc. The report shall show specified quality objectives, the characteristics of the method and whether the method satisfies the quality objectives or: "how good must the method be, how good is it and is it good enough?"

Below please find a list of quality objectives and method characteristics for clinical biochemical method of analyses.

#### 6. Quality objectives for examination methods in Clinical Biochemistry.

Below in a non-prioritised order are stated models or components to determine specifications for analytical quality. There may be compliance with most of them.

Quality objectives established by:

- Authorities
- Providers of PT

Published recommendations:

- From national or international experts
- Local Experts

Quality objectives from "states of the art"

- Data from PT
- Data from recent publications

Evaluation of the quality of the method regarding to its influence on clinical decisions:

- Data based on biological variation, especially the intra-individual
- Data based on the decisions of the clinicians

Evaluation of the quality of the method regarding to its influence on specific clinical decisions, based on studies of the "clinical outcome".

## 7. Prearranged resolutions (preconditions)

Before starting the practical part of the validation, it may be appropriate to establish and describe the below mentioned, which at all circumstances must be shown in the report

- The name of the examination procedure (sample material – component) unit (if relevant) and evt. IUPAC / IFCC / DNK – code
- Equipment
- Environmental conditions ( temp., humidity, requirements regarding to DNA examinations)
- Patient samples, control material; accuracy and imprecision
- Calibrator
- Criteria and requirements for approval/rejection for the maximum accepted uncertainty
- Specifications from the requester
- Biological reference intervals
- Safety and environment

## 8. Method characteristics for the quality of the measurement

It is not possible to state a firm procedure for all analyse methods, therefore choice of method characteristics must be evaluated from test method to test method. Specific method characteristics are mentioned in 17025 [5.4.5.3] and ISO 15189 [5.5.3] and by elaborating the validation protocol these are chosen, supplied and measured according to demands and relevance

### 8.1 Metrological traceability

Definition: Property of a measurement result or measurement standard whereby the result can be related to a stated reference (national or international ) through a documented unbroken chain of comparisons, each contributing with an known measurement uncertainty (6,7)

The top of the calibration hierarchy shall be given. This hierarchy will often start with a SI-unit or other measurement unit and/or a method and/or a calibrator and maybe cross several similar levels to the routine procedure and the result.

### 8.2 Trueness and comparison of methods

Trueness: closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value (8)

The trueness of measurement can be evaluated by

- Analysing a certified reference material or similar
- Recovery test
- Comparison with measures made in other laboratories, applying the same or a different method. The applied method, which is compared to, ought to be approved, well documented and the sample material to be sample from a patient, if possible.

If an important clinical bias is identified, the laboratory shall investigate possible arrangements and correction.

### 8.3 Measuring interval

Definition: a closed interval of possible values that are acceptable values measured by a given measuring procedure, with a lower and a higher measuring limit, and with specified maximal measurement uncertainty, (modified 7)

The measuring interval may be relevant for the needs of the clinicians. In practise the field of measurement is often shown as a primary interval – covered by the calibration curve – and an extended interval which appears by manual or mechanical dilution, respectively up concentration of the sample material. In these cases it must be documented by measurements including calibration also in the extended field that the measurement values have an uncertainty less than the maximum value. The functional sensitivity (defined as CV by the intermediate precision of i.e. less than 20 %) may participate when fixing the lower part of the measuring field.

For method, in which CE-marked reagents and calibrators are used, the manufacturer is obliged by law to give information about the measuring interval and the related maximum measuring uncertainty.

### 8.4 Limit of detection

Definition: Measured quantity value, obtained by a given measurement procedure, different from 0, for which the probability of false negative result is  $\beta$ , given a probability  $\alpha$  of false positive result (9)

The limit of detection is to be determined – if relevant – by repeated measures of patient samples with very low value of measure, subsidiary by repeatedly measures with the 0-calibrator. The concentration corresponding to the measuring average + 5 SD will be a practical useful target for the detection limit, as it is defined above.

### 8.5 Analytical specificity

Definition: Capability of the measuring method to measure the value, alone ( 7 modified)

Materials with well known content of related components (cross reactions testing) or potential interfering components can be tested.

### 8.6 Linearity

Definition: The direct proportionality between measuring value and value of the measuring size within the measuring interval.(10)

For instance, linearity can be validated by using a dilution row made of samples with known concentration close to higher respectively lower limit of measurement and mixed differently.

### 8.7 Intermediate precision

Definition: closeness of agreement between independent measurements in a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects but may include other conditions involving changes (11)

The intermediate precision ought to be estimated by representative concentrations within the measuring interval. Concentrations close to clinical limits of decisions are especially important. The testing can be carried out on patient samples or proficiency testing materials. It is essential that the test, in order to be representative, must take place over a longer interval of time to get the important elements in the day-to-day variation.

If more measuring system and / or operators are used free of choice, these sources of variation must be included.

### **8.8 Robustness**

When validating a method robustness, it is important to cause changes in external conditions, which could have influence on the measurement i.e. change of reagents batches or calibrator batch, temperature and operator. The size of observed nonconformities ought to be recorded but in practice a complete evaluation of the robustness of the method will not be achieved within normal validation time. If the method is CE-marked according to the IVD-directive (as a part of a system for in vitro-diagnostic) the validation of robustness ought to be based on the information from the diagnostic manufacturer.

### **8.9 Carry-over**

Carry-over can be divided in reagent carry-over and sample-carry over. Reagent carry-over is especially seen at “random access” equipment and is often described by the diagnostic manufacturer. Because of the numerous possibilities of combinations, this type of carry-over is often not detected before the equipment is used for the first time.

Sample carry-over is normally seen at batch analysing and can be tested by measuring patient samples with low and high concentration in a defined order.

### **8.10 Pre-analytical conditions**

The validation may include pre-analytical conditions, which can have influence on the measuring results i.e. preparation of the patient, sampling, handling and transportation.

### **8.11 Uncertainty budget**

The objective of the uncertainty budget is to inform the users of the total uncertainty associated with a given value and to show the laboratory how much each factor contributes to the total uncertainty and therefore focus area for improvement of the total quality.

The uncertainty budget must cover all known important contributions to the uncertainty. These can be grouped in order to make the calculation simpler (12). Normally the following elements will be present:

- 1) uncertainty components at pre-analytical conditions
- 2) the uncertainty associated with the determination of the value of the calibrator
- 3) the uncertainty associated with bias-corrections
- 4) the uncertainty of the measurement (normally the intermediate precision)

If experimental data can not be established in a reasonable way, the budgets should be based on valid estimates.

As many as possible of the important uncertainty components shall be taken into account in the budget.

*Therefore until further notice, DANAK find it sufficient that the uncertainty budget only covers the items 2, 3 and 4 in those circumstances when solid data for uncertainty for the pre-analytical conditions are not easily available*

The intra-individual biological variation (13) may be relevant at evaluating the measuring result, but can be stated separately to the clinicians. Please note, that available figures for intra individual biological variation only covers healthy individuals and it is often unclear, whether the sampling uncertainty is included in the figures.

## 9. Conclusion

The report is finished by an assessment of the results and a conclusion whether the method can be approved for use in the laboratory.

## 10. Referencer / References

- (1) DS/EN ISO/IEC 17025: 2000: Generelle krav til prøvnings- og kalibreringslaboratoriernes kompetence.
- (2) DS/EN ISO 15189: 2003: Medicinske laboratorier – Særlige krav til kvalitet og kompetence.
- (3) Bekendtgørelse om medicinsk udstyr til in vitro-diagnostik. BEK nr. 1171 af 17/12/2002
- (4) SICLI 1999; 59 585: Consensus agreement. D. Kenny, C. G. Fraser, P. Hyltoft Petersen, A. Kallner
- (5) NA Dok. nr. 48a Klinisk Kjemi; 2001
- (6) DS:2344: 1995: Metrologi, terminologi – Grundlæggende og generelle begreber-(6.12)
- (7) René Dybkær: Vocabulary for Use in Measurement Procedures and Description of Reference Materials in Laboratory Medicine; Eur J Chem Clin Biochem 1997; 35(2): 141 – 173
- (8) ISO 3534-1: 1993: Statistics – Vocabulary and symbols – Part 1: Probability and general statistical terms-(3.12)
- (9) DS/ISO 11843 –1: 2003: Detektionsevne – Del 1: Termer og definitioner.
- (10) Egen definition
- (11) DS/ISO standard 5725-2: 1995: Nøjagtighed (korrekthed og præcision) af målemetoder og resultater. Del 2: Grundlæggende metode til bestemmelse af repeterbarhed og reproducerbarhed for en standardiseret målemetode.
- (12) Eurachem CITAC: Quantifying Uncertainty in Analytical Measurement, 2. edition, 2000.
- (13) Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M: Current databases on biologic variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59:491-500 (med tilhørende online version og opdatering af databasen på <http://www.westgard.com/biodatabase1.htm>).

## 11 Entry into effect

This guideline (the Danish version) came into force on 25 February 2005

## 12. Annexes

None