Establishing Reference Systems in Laboratory Medicine on the Basis of ISO/CEN Standards

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Abstract

ISO/IEC has issued several International Standards concerning the concept of traceability in laboratory medicine.

prEN ISO 17511 Metrological traceability of values assigned to calibrators and control materials,
prEN ISO 18153 Metrological traceability of values for catalytic concentrations of enzymes assigned to calibrators and control materials,
prEN ISO 15193 Presentation of reference measurement procedures,
prEN ISO 15194 Description of reference materials
prEN ISO 15195 Requirements for reference laboratories

The concept of traceability provides the most important strategy to achieve comparable measurement results independent of the laboratory and the applied measurement procedure.

The implementation of this concept requires

- the development of further vertical standards by international organisations,
- the development of reference measurement procedures,
- the provision of reference materials,
- the establishing of a global infrastructure which approves reference procedures, reference materials and reference laboratories.

The activities of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Joint Committee on Traceability in Laboratory Medicine (JCTLM) for the establishing of reference systems on the basis of ISO standards are demonstrated in some examples.
In recent years, in the field of laboratory medicine, several important standards concerning the concept of traceability have been published by ISO/IEC and CEN:

prEN ISO 17511 Metrological traceability of values assigned to calibrators and control materials,
prEN ISO 18153 Metrological traceability of values for catalytic concentrations of enzymes assigned to calibrators and control materials,
prEN ISO 15193 Presentation of reference measurement procedures,
prEN ISO 15194 Description of reference materials
prEN ISO 15195 Requirements for reference laboratories

The introduction of reference systems and the concept of measurement traceability provide probably the most important strategy in achieving standardisation in laboratory medicine aimed at comparable measurement results regardless of the method, the measurement procedure (test kit) and of the laboratory where analyses are carried out.

Consequently, the In Vitro Diagnostica Directive of the European Union stipulates that values assigned to calibrators and control materials must be traceable to reference materials and/or reference methods of a higher order.

According to the key document in this series – Metrological traceability of values assigned to calibrators and control materials (prEN ISO 17511) - traceability of a value attributed to a routine sample, a calibrator or a control material is established by a series of comparative measurements using measurement procedures and reference materials in a chain of decreasing hierarchical order. Since each link in the traceability chain contributes to the uncertainty of the result it is advisable to omit as many steps as possible. In terms of metrology it would be ideal to omit all in-between steps of the traceability chain and to measure the routine sample directly by the use of a primary reference procedure; this of course is not feasible.

The complete traceability chain as presented here is valid only for measurable quantities which can have a value expressed in SI units. When primary or secondary calibrators are not available the traceability chain for many measurands in laboratory medicine ends at a lower level, e.g. at the manufacturer’s standing measurement procedure. When a manufacturer detects a new diagnostic marker and defines the measurable quantity by establishing a measurement procedure for this marker, the manufacturer’s measurement procedure will form the top of the traceability chain. Nevertheless, even in this simple situation, the principles of the traceability concept are applicable.

An inevitable precondition for the establishment of traceable results for calibrators and control materials is the specificity of the measurement procedures applied. Results of measurement cannot be traceable when the procedure applied partially detects components which are not consistent with the definition of the measurand.

The implementation of the concept of traceability requires – in addition to the ISO/IEC/CEN standards which are usually of horizontal nature - the development of vertical standards describing in detail how to establish reference systems. Furthermore, a global infrastructure is necessary to establish and maintain reference systems with reference materials, reference procedures and networks of reference laboratories.

For many years, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has been promoting the development of reference systems. Consequently, the IFCC is – in addition to the BIPM and the ILAC – one of the founding members of the Joint Committee of Traceability in Laboratory Medicine (JCTLM). The objective of this committee is …..
The successful implementation of the concept of traceability as described in the ISO/IEC/CEN standards may be demonstrated in two examples:

The measurement of cholesterol in human serum is, because of its potential as risk marker for cardiovascular diseases, one of the most frequently performed procedures in laboratory medicine. The unsatisfactory situation of cholesterol measurement only a few years ago may be demonstrated with the results of a ring trial, which was conducted by approximately 1300 laboratories. Two different samples with unknown cholesterol concentration were distributed to the laboratories and the results were displayed in a YOUDEN diagram. Each dot in this diagram represents the two results from one laboratory, whereby the result for sample A can be read from the abscissa and that for the sample B from the ordinate. A laboratory with its dot just in the middle of the screen is in full agreement with the target value, which here is the reference method value certified by isotope dilution mass spectrometry. The participants’ results from this survey for cholesterol in 1987 clearly show that three different groups of data have been reported according to three different methods of cholesterol determination. The participants with relatively high cholesterol results had used the Liebermann-Burchard procedure, which was still in use in 1987. The group with low cholesterol values had applied the cholesterol oxidase iodide method, and the data of laboratories using the CHOD/PAP method are situated in the middle of the screen. In 1987, participants’ results were evaluated by comparison with the means of their peer group according to the different methodological principles used. Differences of up to 50% between the peer group target values could be observed for cholesterol measurements. In view of the fact that there can be only one true cholesterol concentration value in a serum, this situation was clearly untenable.

After introduction of the reference procedure values for cholesterol, based on IDMS measurements, the different peer group target values have now been replaced by one reference method value, which in our case is represented as the exact middle of the screen. The corresponding limits of acceptance are shown as the solid square. As a consequence, methods with inherent systematic errors, like the Liebermann-Burchard method and the CHOD-IODIDE method, disappeared from the market and today only methods exist which are within the limits of acceptance with the reference method values established by isotope dilution mass spectrometry. Thus, comparability of cholesterol results is greatly enhanced by the implementation of the concept traceability.

About 10 years ago it seemed unlikely that a traceability chain for enzymes could be developed to the top level. Enzymes are proteins which catalyse chemical reactions in living cells; they are measured in human body fluids for detection and monitoring of various diseases. In contrast to other measurands in clinical chemistry, like electrolytes or substrates and metabolites such as creatinine or cholesterol, enzymes exist in multiple forms with respect to their chemical, conformational and glycosidic structure. The catalytic activity strongly depends on the measurement condition, e.g. the temperature.

In this situation, the strategy for introducing traceability has to be different. First, it is necessary to find an agreement on the definition of measurand; second, a reference measurement procedure should be developed and established in networks of reference laboratories; finally, reference materials may be developed.

Despite these drawbacks, a strategy for establishing a reference system for enzymes has been developed in the IFCC committee for enzymes which comprises:
- the definition of the measurands by the development of reference procedures,
- the establishing of networks of reference laboratories and
- the provision of reference materials.

The decision on primary measurement procedures was the first objective of the IFCC enzyme committee and a group of expert laboratories for the implementation of the reference system.

Primary reference measurement procedures for ALT, AST, CK, GGT and LD have now been published. A document for Amylase is currently being prepared for mail ballot and publication. The development of procedures for AP, Lipase and CHE is projected.

Results from an external quality control ring trial obtained just before introduction of the IFCC reference system demonstrate the unsatisfactory situation e.g. for GGT-measurements. Obviously, several different measurement procedures existed. This is shown by the separate clouds in the Youden diagram. The overall coefficient of variation was in the order of 26%. It proved necessary to apply different procedure-
dependent target values and different limits of acceptance for the laboratories using different tests. For the patient samples this means that results are not comparable from one laboratory to the other.

The first ring trial after introducing the IFCC reference system in April 2003 demonstrates a considerable improvement of comparability of test results. The overall coefficient of variation decreased from 26 to 9% from one ring trial to the next. The participants' results are now evaluated only on the basis of the IFCC reference procedure target value and the corresponding limits of acceptance. When results from tests of individual manufacturers are displayed separately it becomes obvious that still slight – but acceptable – differences remain.

It can be summarised that after introduction of the IFCC reference system in clinical enzymology results of patient samples will be traceable to the highest metrological level. As demonstrated here, this will considerably improve comparability of test results independent of the individual test kit applied and independent of the laboratory where such clinical chemical testing is performed.

The IFCC enzyme reference system may be regarded as a pilot project for improvements in other fields of laboratory medicine where traceability is currently achieved only on a very low metrological level.

In contrast to enzyme activities, the definition of the proteo-hormone measurand as well as of many tumour markers is highly critical and several aspects have to be regarded as it concerns the sub-unit to be measured (ß-chain or complete molecule), the epitope to be detected and, finally, the glycosidic structure of the molecule.

In view of this, it is not surprising that we find a large scatter of test-kit dependent results in ring trials for Prolactin. The data extend by a factor of about 3 between the lowest and highest reported values. Undoubtedly, the individual tests detect different molecular entities of Prolactin. In this case, different measurands were determined, which, in fact should have different names, e.g. Prolectin-A, Prolactin-B and C.

Accordingly, as long as a global agreement on one particular iso-form is missing, each of these different measurands – despite the fact that they share the same name – has to be judged separately. Consequently, different test-kit specific target values and limits of acceptance have to be applied for the judgement of participants’ results in external quality control.

**In summary**, it can be stated that the concept of traceability and the tools for its implementation are now available for SI-traceable measurands.

For the development of reference systems for non-SI-traceable quantities the predominant objective must be an agreement on the definition of these quantities on an international basis before reference measurement procedures can be developed and used for assigning target values in external quality assessment.