Traceability, reference systems and result comparability

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ABSTRACT

The standardization of measurements is of high priority in Laboratory Medicine. At present, the international cooperation is involved in developing reference measurement systems (reference methods, reference materials, and reference laboratory networks) for measurands of clinical significance to reduce whenever possible uncertainty and to promote comparability of results for a better reliability of the information obtained from routine procedures. The implementation of the measurement traceability to the reference system actually provides one of the most important tools to support standardization in Laboratory Medicine aiming at result comparability regardless of the measurement procedure (test kit) and the clinical laboratory where analyses are carried out. The aim of this review is to discuss some concepts related to the achievement of standardization by the implementation of a metrologically correct measurement system, providing some examples to illustrate the complexity of this approach and the impact of these activities on patient care.

BACKGROUND

The primary goal of Laboratory Medicine is to provide information that is useful to assist medical decision-making, allowing optimal healthcare (1). This can only be obtained by generating reliable analytical results on patient samples. Meaningful measurements are indeed essential for the diagnosis, monitoring, and treatment of diseases, and for risk assessment of individuals. Inadequate laboratory performance may have extensive consequences for practical medicine, healthcare system, and, last but not least, for the patient. Poor-quality results may actually lead to incorrect interpretation by the clinician, impairing the patient's situation.

Foremost among the laboratory problems is the poor comparability of analytical results, especially when they originate from different laboratories using different methods. Nowadays, considerable differences can still be observed in the results of different measurement procedures for the same analyte (2-4). Analytical systems give results that are typical of a particular method or instrument, so that different results from different assays and platforms may be obtained for a given analyte. Such situation may cloud interpretations of reported data, creating a substantive problem for both clinician and laboratory communities.

In 2002, the Institute for Reference Materials and Measurements (IRMM) of the European Union (EU) surveyed 950 global laboratories in an International Measurement Evaluation Program (IMEP) for the measurement of the most common biochemical constituents in human serum (5). Just as an example, results for γ-glutamyltransferase, one of the most commonly employed biomarkers in hepatology, showed biases of -60% to +30% at a serum catalytic activity of 35 U/L, a value close to the upper reference limit. It is easy to argue that, on the basis of this variation, many of the study participating laboratories would misclassify patients at this critical decision level.

Most importantly, the lack of result comparability from different assays causes the inability to define common reference intervals or decision limits for a particular biomarker, thus creating confusion among clinicians when results are interpreted (6). Typical is the case of cardiac troponin I measurement (7). The achievement of the standardization of laboratory measurements, assuring interchangeability of results over time and space, would therefore significantly contribute to improvements in healthcare, since results of clinical studies undertaken in different locations or times could be universally applied (8). This would allow an effective application of evidence-based medicine, as guidelines established by scientific or professional bodies often advocate use of specific decision limits for diagnosis and therapeutic intervention. Using the example of cholesterol testing in the evaluation of cardiovascular risk, data from the U.S. Government Accounting Office have shown that the marked improvement in the accuracy of its measurement in the last 40 years has saved ~100 million dollars per year in treatment costs with a parallel significant reduction of untreated "false-negative" individuals at high risk.

The recognition that it is standardization of results that requires improvement in Laboratory Medicine rose in the recent years questions about the causes for the lack of standardization (9, 10). It was recognized that an insufficient calibration approach, i.e. the lack of result traceability to certified standards, is the most important cause. Consequently, an international agreement on the need to improve standardization through the implementation of metrologically correct measurement systems has been reached (11). The importance of the metrological principles has been described in two documents of

the International Organization for Standardization (ISO), the ISO 17511 and the ISO 18153 (12, 13). In these documents, the traceability to internationally recognized and accepted reference materials and measurements is considered the key element in assuring the accuracy and comparability of clinical laboratory measurements. The EU directive on in vitro diagnostic (IVD) devices supports these ISO standards and requests applications of the standards for all IVD reagents used within the EU with the aim to ensure that the use of IVDs do not compromise the health and safety of patients, users, and third parties and attain the performance levels attributed to them by their manufacturer (14). This European legislation has, however, an obvious worldwide impact. Practically, diagnostic manufacturers must ensure that the analytical systems they market have been calibrated against available certificate reference materials and reference measurement procedures and that repeatability and reproducibility of their internal calibration procedures are quantified and documented (15).

THE CONCEPT OF REFERENCE MEASUREMENT SYSTEM

In order to achieve standardization, an approach that would provide reliable transfer of the measurement values of an uppermost hierarchical level to methods which are routinely used in the clinical laboratories is then needed. Such a structure is presented by the reference measurement system, based on the concepts of metrological traceability and of hierarchy of analytical measurement procedures (11). Key elements of the system are the reference measurement procedure and reference materials. The reference procedure is used to assign a certified value to a given reference material. Once the appropriate reference material is certified, this material and the manufacturer's testing procedure can be used in industry to assign values to commercial calibrators. Clinical laboratories use routine procedures with validated calibrators, both from commercial sources, to measure human samples. In this way, the obtained value will be traceable to the reference procedure and materials, and the standardization of measurement, that is, the process of realizing traceability, will be reached (16).

It should, however, be noted that the practical implementation of the reference system concept cannot compensate for poor precision and lack of analytical specificity of commercial assays. Furthermore, the above statements are only true if the reference materials used to transfer trueness to the field methods are commutable. Commutability is the ability of a reference or calibrator material to show interassay properties similar to those of human samples (17). In practical terms, the numerical ratio between the results determined by a given routine and a reference procedure found for the reference material must be the same as the average ratio found for patients' samples. Only commutable materials can be used by industry for direct calibration of commercial methods, having large importance to ensure an unbroken traceability chain. It is well known that purification procedures sometimes used in the preparation of reference materials may result in non-commutability of these materials with native samples (18). Pure compounds prepared by recombinant techniques may also have altered structures with the consequence that the probability of matrix effects is high (19).

A solution to the commutability problems is the preparation and use of secondary reference materials as an intermediate step in the traceability chain (20). In their preparation, human serum (or defibrinated plasma) is the preferred base matrix, the effects of the natural variation between donors being minimized by using pooled collections from a number of individuals (21). However, although matrix-based materials are desirable as they are more likely to behave in a similar fashion as human samples, this does not a *priori* eliminate the non-commutability problems (22). Thus, also "patient-like" reference materials should be used for calibration of commercial methods only if their commutability has experimentally been proven (8). If commutable reference materials suitable for direct use in the field method calibration are lacking, the only possible alternative for establishing traceability to a reference measurement procedure is for IVD manufacturers to split human fresh samples with a laboratory performing the reference measurement procedure and calibrate the commercial system in accordance with obtained correlation results (23).

In addition to reference procedures and materials, essential elements of a comprehensive reference measurement system also include the definition of the measurand in regards to the intended clinical use and the individuation of reference laboratories that possibly collaborate in a network (Table 1) (24, 25). The main responsibility of reference laboratories is to assign target values to reference materials, using the reference measurement procedures. In addition, as reported above, they may assist commercial companies in the validation of routine procedures through direct comparison of a routine analytical system with the reference procedure, using a number of appropriately selected, native human samples (26). Finally, reference laboratories may be regarded as a concerted means of supporting External Quality Assessment Schemes (EQAS) by setting up reference methods for their control materials in the post-market vigilance of clinical laboratory performance. Once again, this practice should not, however, be endorsed if the commutability of quality control materials between the reference method and the routine procedures for which they will be used has not been validated (27).

Table 1
Components of a working reference measurement system

- Clear definition of the analyte to be measured in the human samples;
- Reference measurement procedure(s) which specifically measures the analyte as being defined;
- Primary and secondary (commutable) reference materials;
- Reference measurement laboratories, possibly collaborating in a network.

As stated before, a detailed definition of the quantity to be measured constitutes an indispensable part of any analytical reference system (28). In Laboratory Medicine, many hundreds different analytes are measured or determined. With regard to the implementation of traceability, it is, however, important to differentiate between:

- analytes which are well defined chemical entities and are traceable to International System (SI) units, called type A quantities, and
- analytes which are rather heterogeneous in human samples and are not directly traceable to SI units, called type B quantities (Table 2).

Type A analytes represent a fair number of well defined compounds (approximately 65), which belong to "classical" clinical chemistry, e.g. electrolytes (e.g. sodium), minerals (e.g. calcium), metabolic products (such as cholesterol, glucose, creatinine, etc.), steroid hormones, and vitamins. Test results of these measurements are nowadays expressed in terms of moles per litre, which represent the accepted system of SI units. However, for many hundreds of measurable quantities, designated as type B analytes, e.g. all proteins and glycoproteins – usually measured by some kind of immunochemical techniques – test results are not expressed in terms of SI units, but in terms of arbitrary units, for example: WHO international units or mass units of a preparation belonging to a manufacturer.

Table 2
Traceability and analyte classification

Type A analytes:
– Well defined compounds;
– Concentrations expressed in SI units;
– Results are not method-dependent;
– Approx. 65 analytes (e.g., metabolites, electrolytes, steroid hormones);
– Full traceability chains.
Type B analytes:
– Not well defined (often heterogeneous mixtures);
– Analytes can be bound or in free state;
– Not traceable to SI units, but to arbitrary units (e.g., WHO International Units);
– Immunochemical procedures show inherent variability (different epitopes);
– 400-600 analytes (e.g., tumor markers, viral antigens, clotting factors);
– Full traceability chains frequently not available (calibration on widely used methods).

For type A analytes, reference materials containing the analyte as a pure compound can usually be prepared and reference measurement procedures which specifically measure the analyte and are independent of routine analytical principles can be developed. Consequently, for many of these analytes, reference systems are already available (29). A good example of a reference measurement system for type A analytes is that for creatinine in blood serum (Figure 1) (30). Creatinine is a chemical substance whose entity can be unequivocally defined as single species. The unit for the measurement of the amount-of-substance concentration of creatinine is mol/L and gravimetry can therefore be used for the value assignment of a primary reference material prepared with the pure substance. The reference measurement procedure for creatinine, applying the isotope dilution-mass spectrometry (IDMS) principle, is directly calibrated against this primary reference material. Using this reference procedure, realized in reference laboratories working under well-defined performance conditions, values are assigned to a secondary commutable reference material. Manufacturers then may apply this material for calibration of a routine method, leading to traceable results of the end user's routine method.

For type B analytes, the implementation of standardi-

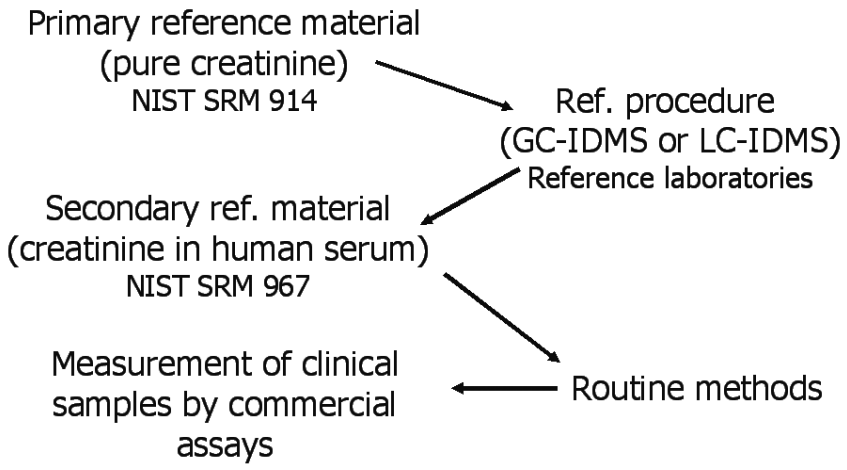

Figure 1
The reference measurement system for serum creatinine. Adapted from ref. 30.
NIST, National Institute of Standards and Technology; SRM, standard reference material; GC-IDMS, gas chromatography-isotope dilution mass spectrometry; LC-IDMS, liquid chromatography-isotope dilution mass spectrometry.

zation is in general more difficult. Much scientific work still has to be done before reference measurement systems for this type of quantities can be established. Since these analytes are heterogeneous and their composition in human body fluids varies, all reference materials for type B analytes are, by definition, only surrogates for the analytes to be measured in patient samples. While such materials may resemble to some extent the typical heterogeneous mixture of the analyte present in the human fluids, it often represents only an "average" condition (24, 31). Furthermore, for type B analytes, reference measurement procedures independent of routinely employed analytical principles are currently lacking in the majority of cases (32). Thus, the value assignment to candidate secondary reference materials is frequently problematic (33). As a consequence, manufacturers prepare their own calibrators and assign values on a mass basis of the chosen preparation that is often not available to others, leading to a disagreement between results from different commercial assays.

As many type B analytes are very important parameters in the medical field, such as oncology, endocrinology, and virology, the establishment of a reference measurement system for these measurands is, however, urgently needed. For these analytes also, the traceability model emphasizes the importance of the definition of the measurand. When considering complex substances, the definition may indeed not be so clear, because of their potential intrinsic or acquired heterogeneity. As a matter of fact, the heterogeneity of type B analytes may be circumvented by the definition of a unique, invariant part of the molecule that is common to all components of the mixture present in blood. Methods used for the development of commercial assays should, without distinction, recognize this common part with a consequent increase in the homogeneity of assay reactivity. According to this approach, in recent years a number of significant efforts have been initiated to standardize measurement results for type B analytes.

An excellent example is the IFCC project for standardization of measurement of haemoglobin A1c (HbA1c) (34). In the specific Working Group the decision was made to define HbA1c as hemoglobin molecules having in common a glycated amino-terminal hexapeptide of the hemoglobin β-chain. The rationale was that this quantity is biochemically well characterized, is the major form of HbA1c in human blood, and most of the commercial tests claim to measure only this form. Two equivalent reference measurement procedures specifically measuring this hexapeptide were then developed, with a combination of high-pressure liquid chromatography (HPLC) and electron-spray mass spectrometry or, alternatively, a two-dimensional approach using HPLC and capillary electrophoresis (35). Finally, secondary reference materials have been prepared and their HbA1c values certified by a network of reference laboratories, allowing the establishment of a complete reference measurement system (Figure 2) (36).

A special class of analytes are enzymes, defined in terms of the amount of an agree-upon substrate they convert in an agreed-upon measurement system, the so-called "catalytic amount". Theoretically, enzymes defined by substrate conversion do not belong to the SI-analytes, even if the definition of "katal" may suggest so (37). It is namely a fact that the analyte may well be part of a family and, in some cases, may be totally or partially unknown (24). Hence, the problems of mixture analysis and unknown entity, typical of type B analytes, may also apply for enzymes defined by substrate conversion.

Oppositely to other analytes, the numerical results of catalytic activity measurements depend entirely on the experimental conditions under which the measurements are made (13). In the standardization of enzyme assays, therefore, a reference measurement procedure, which defines the conditions under which a given enzyme activity is measured, occupies the uppermost position filled, with regard to non-enzyme analytes, by primary referen-

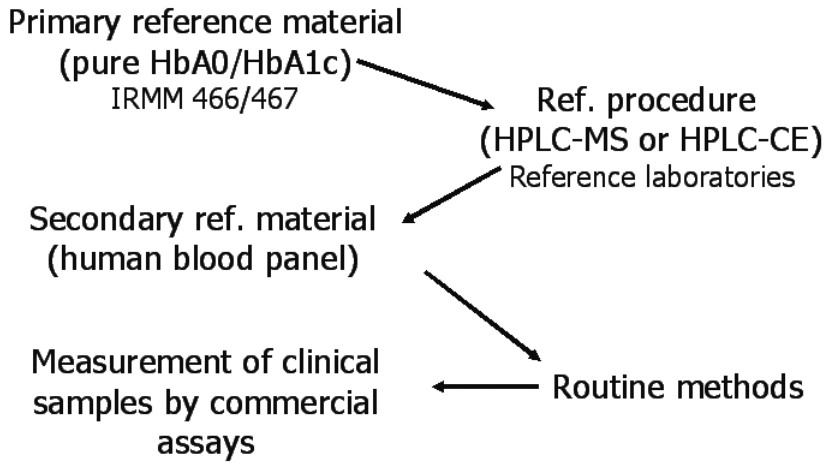

Figure 2
The reference measurement system for haemoglobin A1c (HbA1c).
Hb, hemoglobin; IRMM, Institute for Reference Materials and Measurements; HPLC-MS, high-pressure liquid chromatography-mass spectrometry; HPLC-CE, high-pressure liquid chromatography-capillary electrophoresis.

ce materials (38). Complete reference measurement systems, comprising reference measurement procedures, reference materials, and reference laboratories, are currently available for alanine aminotransferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, and amylase. For aspartate aminotransferase the certification campaign of the reference material using the IFCC reference procedure is ongoing. Reference systems for alkaline phosphatase and pancreatic lipase are also under discussion.

THE INTERNATIONAL COOPERATION

Since the development of metrologically sound reference measurement systems is a complicated and expensive process, it is clear that the objective of improving standardization in Laboratory Medicine will only be achieved if the problems are dealt with not on a national level but through international cooperation. In order to avoid confusion and waste of resources, the development and establishment of different national or regional analytical reference systems are not acceptable. International consensus and agreement should be reached by all major players in the field. This was the reason for the creation of the Joint Committee on Traceability in Laboratory Medicine (JCTLM), established under the auspices of the International Bureau of Weights and Measures (BIPM), the IFCC, and the International Laboratory Accreditation Cooperation (ILAC) (39). In addition to these international and intergovernmental organizations concerned with measurements in Laboratory Medicine, metrology, and health, other JCTLM key stakeholders are represented by the principal producers of IVD reference materials; the IVD industry associations from Europe, Japan and the US; regulatory bodies from Europe, Japan and the US; standards writing bodies, and accreditation and quality assessment organizations.

Since April 2004, a list of higher order reference materials and methods for analytes measured in Laboratory Medicine, identified by a thorough review process for conformity with appropriate ISO standards, is publicly available in a database at the BIPM website (http://www.bipm.org/en/committees/jc/jctlm/jctlm-db). JCTLM is also publishing the initial list of reference laboratories that fulfil the established selection criteria and are able to deliver a reference measurement service. Using these validated reference measurement systems, industry can assign traceable values to commercial calibrators. Clinical laboratories, which will use routine procedures with these validated calibrators to measure human patient specimens, may finally obtain comparable results. Then, the traceability requirement, as formulated by the IVD directive of the EU and in the corresponding ISO standards, can finally be implemented in practice (Figure 3).

METROLOGICAL VS. "CLINICAL" TRACEABILITY

As soon as a new reference measurement system is adopted and implemented, clinical validation of the correctly calibrated routine methods (the IVD products sold onto the market) should take place. In specific cases, in order to maintain the value of clinical experience, correlation of measurement results obtained with the new cali-

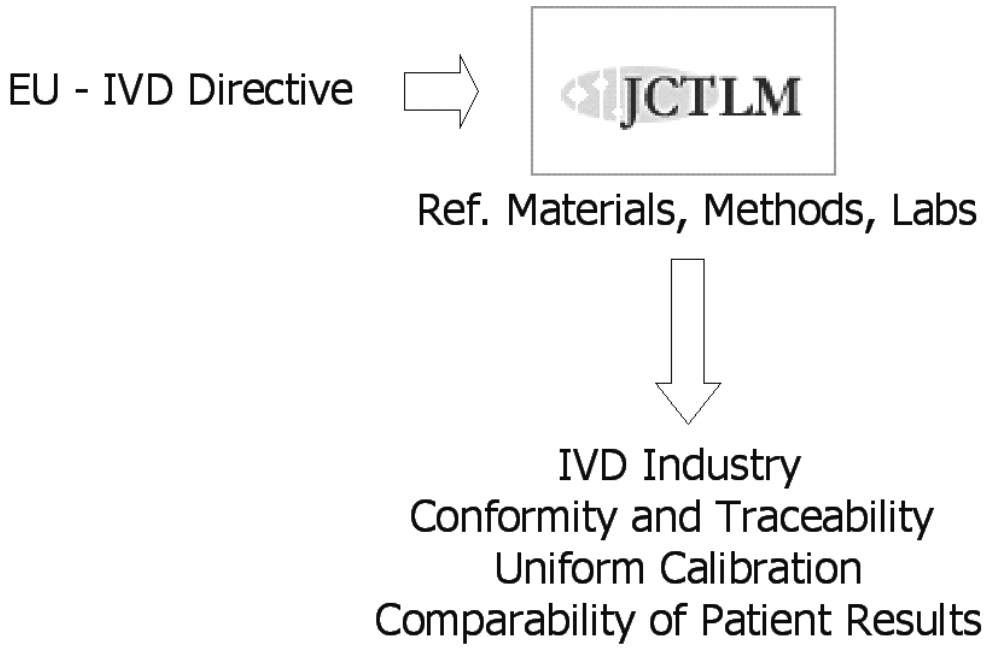

Figure 3
The Joint Committee for Traceability in Laboratory Medicine (JCTLM): the link between the implementation of the in vitro diagnostic (IVD) directive of the European Union (EU) and standardization in Laboratory Medicine.

bration to results of measurements obtained with the previous calibration should be established. Adjusting the decision-making criteria is of outstanding importance since, even if from a metrological point of view the routine method was biased, clinicians can still reach correct clinical decisions if the decision-making criteria they apply incorporate the same bias. In contrast, they could arrive at incorrect clinical decisions if patient results are true with regard to the reference system, but the decision-making criteria are only valid by using the previous calibration for the test.

Referring to the HbA1c example, reliable, linear relationships between results traceable to the IFCC reference system and previous routine methods were demonstrated allowing the conversion of analytical and clinical data from one system to another (40). It is therefore practically possible to translate target values generated in previous landmark clinical studies, using methods not traced to IFCC system, in order to maintain the clinical experience. In addition, the use as the unit of measurement for HbA1c of "mmol/mol" that represents the SI unit for this analyte can be very useful to avoid confusion in the recalculation of the old HbA1c targets to the new IFCC standardized results if clinical laboratories wish to implement HbA1c results traceable to the IFCC reference system (Table 3). Other advantages of this approach may include a positive impact of changing of scale of reported HbA1c results allowing clinicians and diabetic patients to better understand the analyte changes (currently they may perceive small changes – although linked to large health effects – as unimportant) and increased potential for future use of HbA1c as diagnostic tool.

STILL UNDEFINED ISSUES

Considering the implementation of a metrologically correct approach for result standardization, there are still some important undefined issues. First, a clear definition of the clinically justifiable error of measurements is required. Since methods with a total error of zero do not exist, it has to be agreed what percentage of misclassification of patients is acceptable and whether false positive or false negative classifications should be avoided preferably. Whereas statistical validation criteria for analytical performance can be easily defined, tolerable deviations for clinical use are often undefined. As underlined by Thienpont et al. (16), the scientific community has to be aware that the absence of specifications derived from clinical needs for validation of metrologically traceable calibrations might results in a large gray zone with respect to the extent of traceability expected from IVD manufacturers, partially or totally invalidating its theoretical advantages.

The second important issue relates with the post-market vigilance of the performance of IVD medical devices (2, 4). This should be one of the major tasks of our profession through the organization of appropriate EQAS or proficiency testing. The applicability of the true value concept in EQAS require, however, the availability of target values assigned to control materials by laboratories using reference methods and that these materials behave exactly as human patient specimens (41). True value assignment to commutable EQAS materials will allow an objective evaluation of the performance of IVD devices, together with an accuracy-based (instead of inferior consensus-group) grading of competence of participating clinical laboratories (42).

Table 3
Suggested units and target values for hemoglobin A1c when measured with methods traceable to the IFCC reference system. A comparison with the current figures is also given

	Current[a]	IFCC traceable methods
Reference interval (non-diabetics)	4-6%	20-42 mmol/mol
Target for treatment in diabetics[b]	<7%	<53 mmol/mol
Change of therapy in diabetics[b]	>8%	>64 mmol/mol

[a]refer to methods aligned to the U.S. National Glycohemoglobin Standardization Program.
[b]as recommended by American Diabetes Association.

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