TRACEABILITY IN LABORATORY MEDICINE

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The term "traceability" has become a familiar concept to experts in metrology, laboratory medicine and regulatory affairs, assisting in writing ISO/CEN documents. However, to many outside this group it still is a buzzword. This short paper deals with "traceability" and what may be expected from implementing it.

From the outset, it should be made clear that the term "traceability" means, in the context of laboratory medicine, metrological traceability, i.e. pertaining to measurement and measurement results and is applied to in-vitro diagnostic (IVD) systems. Why has this term – metrological traceability – appeared so prominently? The EC Directive on in-vitro diagnostic medical devices mentions the term as one of the essential requirements (1). The full text reads: " the traceability of values assigned to calibrators and control materials must be assured through available reference measurement procedures and/or available reference materials of higher order".

When a calibrator in a IVD product has been assigned a value of X mmol/L for quantity (analyte) A and Y IU/L for quantity (substance) B, how then did the manufacturer arrive at the figures X and Y respectively in terms of which reference material and through which measurement procedure?

What essentially is asked for is that a Reference Measurement System (RMS) should be considered and if not available should be set up for each and every quantity measured or determined in Laboratory Medicine. Such system comprises a Reference Measurement Procedure (RMP) and a Reference Material (RM). The analytical validity of such system should be checked by what is now called a network of Reference Measurement Laboratories (2).

The first question might be: who is responsible for implementation of the traceability requirement? The answer is simple: the manufacturer of IVD products. Not only for the European market, but also to their worldwide or global market.

The text on the traceability requirement, quoted above, raised questions of what was exactly meant and how this should be interpreted. The EC commissioned CEN to prepare a document on this topic (3); during its course of development, ISO entered the scene, and eventually it resulted in a combined document, EN ISO 17511 (4). This document has now been adopted. It explains the concept of metrological traceability of measurements in biological fluids, and details the (im)possibilities of traceability of values.

Importantly, the document is underpinned by a coherent and hierarchical set of definitions (terminology) that applies to the daily practice of Laboratory Medicine (5). This set of definitions is mainly based on definitions developed by ISO working groups over the past years. It is advocated that this set of definitions is adopted and used by manufacturers, users and regulatory agencies; eventually it will greatly improve communication.

What is the goal of the EN ISO Standard on traceability?

Goal: to improve the comparability of test results over time and space (6). This is a lofty aim, but not a new one; many years ago Tietz (7) advocated that more efforts should go into 'standardisation' of laboratory tests, and recently Müller (8) discusses reference measurement systems.

Prerequisite: to achieve the above aim, it is of paramount importance to define the quantity (analyte/substance) to be measured in the chosen biological fluids, and once the definitions of the possible components have been agreed upon, to assess their clinical relevance.

Quantitative / Qualitative

It is well known that most examinations done in Laboratory Medicine are quantitative measurements, except for example blood group tests. By many, manufacturers and professionals in laboratory medicine, some tests are also referred to as "qualitative" (yes/no answer or +, ++, +++ answer). This is a misconception as the so-called qualitative tests actually all are quantitative: the cut-off point chosen is related to a certain concentration of the quantity. To complicate matters further, a concentration above the cut-off point is often referred to as a 'positive test result' and below the cut-off point as a 'negative test result'.

It should be noted that they are interpretations of the test result, and are not the outcome of the 'measurement'.

Traceability to SI and arbitrary units: in laboratory medicine many hundreds of 'measurable quantities' (also called 'analytes') are measured or determined. A fair number (~ 100) belongs to 'classical' clinical chemistry e.g.

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electrolytes/metals, metabolic products (cholesterol, urea, uric acid etc), steroids, thyroid hormones (T4 and T3) and vitamins. Test results of these measurements are nowadays expressed in terms of (fraction) moles per litre, i.e. in the accepted system of SI units.

For convenience, this group is designated as 'type A' and its quantities called analytes.

However, for many hundreds of measurable quantities (estimated at ~ 500) e.g. all proteins and glycoproteins – usually measured by some kind of immunochemical technique – 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.

For convenience, this group is designated as 'type B' and its quantities called substances rather than analytes (9).

Having thus made the important distinction between SI-traceable analytes and non-SI traceable substances, the legitimate question arises whether for these analytes/substances reference measurement systems (RMSs) are available.

Availability of Reference Measurement Systems

In the clause on 'traceability' in the Directive the term availability of procedures and/or materials is used. It is one of the crucial points in this requirement. Are reference measurement systems available for all quantities (analytes/substances)?

For the type A analytes the answer is yes, in many cases (Table 1). However, the implementation thereof is sometimes lacking. Examples: for many type A analytes, an internationally agreed reference material is available, mainly through the US National Institute of Standards and Technology (NIST). Furthermore, reference measurement procedures – independent from the routine measurement procedures, i.e. the commercially available products – are also available e.g. mass-spectrometric techniques.

Not in all cases, have the RMSs been properly set up and not yet been validated by reference measurement laboratories (2). It may be argued by experts in the field of type A analytes that there are problems e.g. total electrolyte vs. ion-activity measurements, bilirubin etc.. International agreement should and could be sought for these problems.

However, these problems are minor in comparison to the problems arising with the type B substances.

Table 1
Reference Measurement Systems for SI-traceable analytes

Ref. Meas. Procedure	Ref. Material	Number of analytes
+	+	~ 60
(+)	(+)	~ 40

( ) indicates potential availability

For the type B substances, the answer to the question of availability of RMSs is a firm no, with a small number of exceptions.

Type B substances are, for example, all proteins and glycoproteins, usually determined or measured by means of immunochemical techniques. Many of these substances have a defined biological activity e.g. (glyco)protein hormones or coagulation factors, but others do not e.g. CEA or PSA. For many readers it may appear difficult to realise that what is measured as a biological effect (e.g. by in-vivo or in-vitro bioassay) is of a different kind than that measured by binding to an antibody. They may look similar but certainly are not identical.

It will be put forward that for many of these type B substances, reference materials are available: for example WHO International Standards (ISs; reference materials). This of course is true, but what are the properties of such reference materials? The ampoules contain preparations prepared on the biochemical laboratory bench according to state-of-the-art methods; they are claimed to be highly purified but may be still heterogeneous e.g. with respect to glycosylation. It should be realised that during the purification process a certain form or forms may have been selected.

Do we know what is present as 'substance' in the biological fluid e.g. serum, plasma or urine? The answer in 99.9% of the instances, we do not know! How then are we allowed to measure the unknown substance in e.g. serum constituting a highly heterogeneous mixture of intact molecules, isoforms, glycosylated forms and breakdown products, against a reference material that may constitute either a single molecular form or a highly heterogeneous mixture? Well we are not allowed, but have done so for so-called practical reasons for the past thirty years.

The two mixtures may have forms in common but they are certainly not identical.

It is concluded that practically all available reference materials for type B substances are surrogates for the substances we "measure" in the biological fluids chosen.

In the EN ISO 17511 document the WHO reference materials are classified as 'international conventional reference materials'.

Table 2. reviews the current situation with respect to availability of RMP and RM for type B substances. An RMP independent of the routine measurement procedure (i.e usually immunoprocedure) is definitely not available, except for HbA1c.

1 IFCC working group/subcommittee

2 clotting assays (biological effect) with often 'normal plasma' as reference material; also WHO ISs.

The most serious problems then are found with the WHO reference materials (n= ~ 300) and a large number of substances (n= ~ 300) where neither a RM nor a RMP is available. In the latter case, the manufacturer chooses its own reference material and assigns values on a mass basis of the chosen preparation that is often not available to others.

The argument remains howver: is the preparation rep-

Table 2
Reference Measurement Systems for non-SI traceable substances

Ref. Meas.Procedure	Ref. Material	Number of substances	Examples
+	+	~ 10[1]	HbA1c;enzymes
+[2]	-	~ 30	clotting factors
-	+	~ 300	WHO ISs in various fields of application
-	-	~ 300	e.g. tumour markers

[1]IFCC working group/subcommittee
[2]Clotting assays (biological effect) with often "normal plasma" as reference material; also WHO ISs

resentative of what is in the biological fluid of the patient.

Global agreement on the definition of quantity in biological fluids

Obviously to anyone this cannot be solved instantaneously. How can we make progress in this 'minefield'? Certainly, no individual, no individual company, no individual country can claim to possess the expertise and knowledge to solve these problems. International, i.e. global, consensus and agreement should be reached.

Firstly, the manufacturers and the professionals in laboratory medicine, in close collaboration with clinicians specialised in their field of specialty and routinely seeing patients, have to sit down and discuss for each and every analyte or substance "what is the definition of the analyte/substance in the biological fluid"? The task of the specialists/clinicians is to ascertain that with such definition the diagnosis and/or monitoring of disease in patients is maintained or improved.

It should be noted that the definition of the substance for diagnosis may be different from the one for monitoring; a notion that as yet is hardly recognised.

Implementation(10)

Secondly, who then decides in an organised fashion what to endorse, what to do and to decide on priorities and timelines?

Joint Committee on Traceability in Laboratory Medicine (JCTLM): this group was formed in June 2002. Four main 'sponsors' support it: the professionals in Laboratory Medicine (IFCC), in metrology (BIPM/CIPM), in health (WHO) and in accreditation (ILAC); furthermore, representatives of trade associations of the IVD industry take part in this truly global endeavour (EDMA, AdvaMed, JACR), of reference materials providers (EU-JRC-IRMM, NIST,HECTEF), of regulatory bodies (FDA, EU-Commission) and of external quality assurance/proficiency testing organisations (EQALM, CAP). Two working groups were established: number 1. dealing with RMs and RMPs, and number 2. dealing with networks of reference measurement laboratories.

Many of the questions raised above should be addressed by WG1, and it is hoped that they will come up with bold, wise and adequate solutions for establishing RMSs for a large number of substances. Possibly, research will have to be done e.g. to answer adequately the question "what do we want to measure in biological fluids?".

If and when solutions are found, they should eventually be implemented by IVD industry.

Only then have the requirements of the IVD Directive and its supporting document EN ISO 17511 been met. It should, however, be realised that as soon as a RMS is adopted and implemented, clinical validation of the then correctly calibrated routine measurement systems (the IVD products sold onto the market) should take place. It will come as no surprise that possibly reference intervals may have to be changed. It is well known that for many analytes and particularly for substances, the presently used reference intervals are of empirical origin, eventually appearing into textbooks and thus have been 'canonised' without solid scientific foundation.

Uncertainty of measurement

The document on Metrological Traceability (EN ISO 17511) holds another surprise, which is that the manufacturer is obliged to calculate the uncertainty of measurement (11) for the value assigned to the calibrator(s) and to describe it in the product-related technical documentation.

However, in the document, it is shown that the application of the calculation of uncertainty of measurement is extended down to the level of the patient' result; it is noted that at that level the manufacturer bears no responsibility. Should the laboratorian then calculate the final uncertainty of measurement of the value assigned to the patient' sample? Should this be reported to the clinicians? And if so, what will the clinician do with it? The important question arises whether the analytically, scientific expression of uncertainty of measurement will improve diagnosis of and monitoring of therapeutic measures in disease? Some far-reaching questions arise, which at this moment cannot be properly answered because the professionals in Laboratory Medicine, nor the clinicians have discussed the pros and cons of the addition of uncertainty of measurement to the reported values.

It will be clear to everyone that error calculation should be performed within a laboratory serving hospital and general practice clinicians, as well as reported when pa-

tients are monitored for therapeutic measures: effects e.g. T4 replacement therapy, anti-coagulant therapy, chemotherapy.

However, this is done on a local scale with – preferably – the same reagents and instruments.

What the effect will be when uncertainty of measurement is reported in a general way for use by the outside world is fully unknown?

In a few years' time surely articles will appear on this subject.

REFERENCES

1. Directive in vitro diagnostic medical devices (79/98/EC), Annex I Essential requirements, Section A.
2. For requirements see ISO 15195; entitled: Laboratory Medicine – Requirements for Reference Measurement Laboratories.
3. CEN Technical Committee 140 working group 4 (CEN TC 140 WG40 and later on ISO Technical Committee 212 working group 2 (ISO TC212 WG2)
4. EN ISO 17511; entitled: Laboratory Medicine p measurement of quantities in biological samples – metrological traceability of values assigned to calibrators and control materials.
5. Dybkaer R. Vocabulary for use in measurement procedures and description of reference materials in laboratory medicine. Eur J Clin Chem Clin Biochem (1997);35: 141 – 173 (some 150 terms are defined)
6. EN ISO 17511. Introduction
7. Tietz NW. Accuracy in clinical chemistry – does anybody care? Clin Chem 1994; 40 : 859 – 861.
8. Müller MM. Implementation of reference systems in laboratory medicine.Clin Chem 2000; 46: 1907 – 1909.
9. Please note: the term 'analyte' suggests a single compound; the term 'substance' refers to a heterogeneous mixture in of holo forms, isoforms, glycoforms and breakdown products. Sometimes a 'substance' is called a 'biological substance' which has a defined biological activity
10. Acronyms used: AdvaMed – Advanced Medical technology Association, USA; BIPM – Bureau International des Poids et Mesures, Int; CAP – College American Pathologists, USA; CIPM – Comité International des Poids et Mesures, Int.; EDMA – European Diagnostic Manufacturers Association, EU; EQALM – European External Quality Assessment Scheme in Laboratory Medicine, EU; FDA – Food and Drug Administration, USA; HECTEF – Health technology Foundation, Japan; IFCC – International Federation of Clinical Chemistry and Laboratory Medicine, Int; ILAC – International Laboratory Accreditation Committee, Int; JACR – Japanese Association for Clinical Reagents, Japan; JRC-IRMM – Joint Research centre – Institute for Reference Materials and Measurements, EU; NIST National Institute of Standards and Technology, USA; WHO – World Health Organisation, Int.
11. EN ISO 17511, clause 6.