

# Traceability of Measurement Results

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### Summary

- Trueness must be independent of analytical platform and measurements comparable regardless of the analytical procedure used.
  - Traceability requirements for the clinical laboratory are via National Metrology Institutes, Reference (Calibration) laboratories and finally the routine laboratory.
  - Traceability information required by today's clinical laboratory may be requested from the manufacturer of the analytical kits and the internet.
  - Traceable laboratory results will greatly enhance the role of the laboratory in patient management.
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### Introduction

Over the past 20 years medical laboratories have witnessed an exceptional growth of analytical systems providing a very large variety of automated assays. This development has allowed the clinical laboratory to play an integral part in disease diagnosis and management. It follows that analytical methods used by such systems must be true if meaningful results are to be reported. Trueness of a test method is possibly the paramount concern of the clinical laboratory but it is often forgotten that trueness should be independent of the analytical platform and the analytical procedure used.

Unfortunately, different test methods can produce divergent test results, necessitating method-specific reference intervals for their interpretation. This undesirable situation prevents portability of patients' records, realisation of common reference intervals and decision limits, realisation of benefits from international studies and their contribution to evidence based medicine. Despite the concept of standardised results having been pioneered back in the seventies by Tietz<sup>1</sup> and a significant volume of work being published on this topic subsequently, standardisation remains elusive for many methods. However, I believe we have turned the corner mainly due to the enforcement of the European Directive on In Vitro Diagnostic medical devices (98/79/EC)<sup>2</sup> on manufacturers of these test systems. The Directive states that the traceability of values assigned to calibrators and control materials must be assured through available references of a higher order. Only those test methods meeting the requirements of this Directive are allowed to carry the CE mark and may be supplied for

diagnostic use by European laboratories. It is important to note the upcoming legislation on regulation of in vitro diagnostic products supplied in Australia has similar requirements to the European Directive.

### What is traceability?

According to the Vocabulary in Metrology (VIM),<sup>3</sup> measurement traceability is defined as "the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties." Using a normal dictionary, interpretation of the word traceable has a wide range of colloquial meanings, the most appropriate being 'able to be followed to the source'. This adds nothing to the ISO definition, but does highlight two important points. The ISO definition tells us where the chain begins and ends. Specifically, it begins with the measurement result, not with the instrument. Secondly, the uncertainty provides a measure of the proximity of the result to the original source.

Providing support to the Directive, the European Commission of ministers mandated two European (now ISO) standards, ISO 17511 "Metrological traceability of values assigned to calibrators and control materials" and ISO 18153 "Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials".<sup>4,5</sup> These standards describe the acceptable value transfer process from reference materials and/or methods of a higher metrological order to materials and/or methods of a lower order.

To implement the concept of metrological traceability, the International Committee of Weights and Measures (CIPM) represented by the International Bureau of Weights and Measures (BIPM), the International Federation for Clinical Chemistry (IFCC) and the International Laboratory Accreditation Cooperation (ILAC) agreed to form a Joint Committee for Traceability in Laboratory Medicine (JCTLM) with the main objectives of providing leadership in identifying reference materials and reference methods appropriate to meet “higher order” requirements, and in developing protocols for the creation of a clinical reference (calibration) laboratory network.

The JCTLM established two working groups for the realisation of both elements of the traceability chain, i.e. reference materials and methods (Working Group 1, WG1), and for identifying complete functional reference measurement systems that apply the first two components (Working Group 2, WG2). The details on the nominations as well as the approved reference materials and measurement procedures are available on the BIPM website<sup>6</sup> with examples shown in Tables 1 and 2.

### **The Traceability Chain**

The Figure depicts the usual route undertaken by various manufacturers to ensure traceability according to EN ISO 17511 and is a practical way of explaining how traceability of a value is assigned to a sample through value transfer from commercially available calibrators and quality controls.

It is important to note that this traceability chain is only valid for analytes that are expressed in SI units. Most measurements in medical laboratories are relative measurements based on the comparison of patient samples with a reference standard using a selected method of comparison. The comparison is done indirectly through the chemical signals generated by both sample and reference standard within defined measurement conditions. It is critical that the value assigned to reference materials has a link to values obtained by reference measurements or to values carried by a certified reference material which itself is linked to values obtained by a reference measurement. The traceability to an SI unit begins with the definition of the ‘amount of substance’ measured by a primary reference measurement procedure in moles or kilograms, the unit of measurement. The substance to be measured must be well characterised and available in its pure form. There are two types of analytes, i.e. Type A and Type B.

**Type A analytes:** These are physico-chemically well defined compounds that are available in pure form, e.g. electrolytes, urea, glucose, cholesterol, uric acid, etc. and can be expressed in molar units (SI unit). The assigned value of this pure

substance is then transferred to matrix-matched, secondary reference materials through calibration of the primary reference measurement procedure.

**Type B analytes:** These do not represent a uniform substance but consist of a heterogeneous mixture of substances which may differ from person to person as well as within the same person depending on health and disease status, e.g. human chorionic gonadotropin (hCG), tumour markers, cardiac troponin, etc. Pure forms of these mixtures are not available, and therefore primary reference materials of Type B analytes do not exist. Hence, Type B quantities cannot be expressed in SI units. Most of these quantities are expressed in arbitrary units such as IU (International Units), e.g. WHO IU or artificial molar units by referring to reference preparations.

### **Traceability Infrastructure**

The practical realisation of traceability is achieved through establishment of a measurement infrastructure made up of three levels as follows:

#### **Level 1: National Metrology Institutes (NMI)**

Once NMI has demonstrated competence in Key Comparisons it becomes a custodian of SI units. In simple terms this means that the NMI can offer its calibration and measurement capabilities (CMC) for certifying specific reference materials. The competent NMI will be listed in the BIPM Key Comparison Data Base (KCDB) list of National Metrology Institutes with its CMC indicated in Appendix C. This laboratory will be automatically listed under the JCTLM data base of Reference Laboratories.<sup>6</sup>

#### **Level 2: Reference (Calibration) Laboratories**

Reference laboratories operate at a higher metrological level than routine laboratories. The level of the results from Reference Laboratories should be appropriate for medical requirements. These laboratories are also known as expert institutions because they perform measurements with the greatest competence. A laboratory will qualify as a Reference Laboratory if it satisfies the following requirements:

1. Accreditation as a Calibration Laboratory according to ISO 17025<sup>7</sup> and 15195<sup>8</sup>
2. Use of a Reference Method that has been approved and listed by JCTLM WG2
3. Participation in Reference Laboratory Ring Trials.

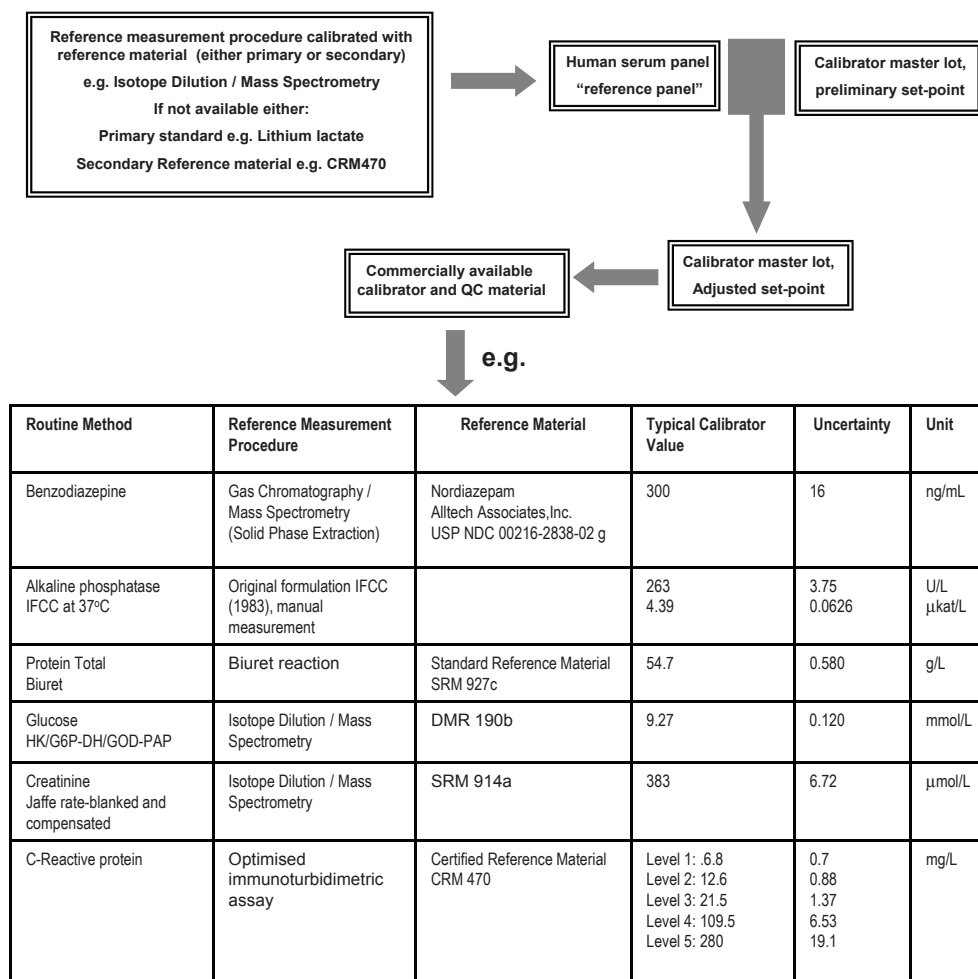
Laboratories that satisfy these conditions will be listed in the JCTLM list of Reference Laboratories. The laboratories that fall into this category offer their calibration and measurement capabilities to Diagnostic Kit Manufacturers, Regulatory Organisations and External Quality Assessment (EQA) Organisations by providing trueness-based values for Ring Trials of Testing Laboratories. NMI and Calibration

Table 1. Examples of higher order reference materials.

|   | Alanine aminotransferase  | Creatine kinase MB (human heart)  | Cholesterol  | Progesterone  |
|---|---|---|--|---|
| <b>Name of the reference material</b>                 | ERM-AD454/IFCC, alanine aminotransferase  | ERM-AD455/IFCC, creatine kinase MB  | SRM 911b, cholesterol  | ERM-DA347, progesterone in human serum  |
| <b>Quantity</b>                                       | Catalytic amount  | Catalytic amount  | Mass fraction  | Amount-of-substance concentration   |
| <b>Analyte certified / Assigned value</b>             | 3.09 µkat/L   | 1.68 µkat/L   | 99.80%   | 10.13 nmol/L  |
| <b>Expanded uncertainty (Level of confidence 95%)</b> | 0.07 µkat/L   | 0.07 µkat/L   | 0.10%  | 0.21 nmol/L   |
| <b>Reference(s) on commutability</b>                  | Commutability for one commercial assay was demonstrated: Clin Chem 2001;47(suppl):A25                                 | Commutability for one commercial assay was demonstrated: Clin Chem Lab Med 2001;39(suppl):S369                        | Not applicable: a high-purity material used as a primary calibrator for higher order reference methods | Not reported  |
| <b>Other relevant publication(s)</b>                  | Certification: Clin Chem Lab Med 2002;40:739-45<br>Procedure used for certification: Clin Chem Lab Med 2002;40:718-24 | Certification: Clin Chem Lab Med 2002;40:739-45<br>Procedure used for certification: Clin Chem Lab Med 2002;40:635-42 | None   | Not reported  |
| <b>Comment(s)</b>                                     | This material was previously named as IRMM-454  | This material was previously named as IRMM-455  | Cholesterol in crystalline material  | The certification of progesterone in two lyophilised serum materials CRM 347 and CRM 348, Report EUR 12282 EN, 1989 |
| <b>Traceability</b>                                   | SI, IFCC reference procedure  | SI, IFCC reference procedure  | SI   | SI  |
| <b>CRM listing</b>                                    | List I  | List I  | List I   | List I  |

**Table 2.** Examples of reference measurement procedures.

| Analyte                        | Reference measurement method / procedure                 | Applicable matrix(s)                                | Measurement principle / technique                         | Reference(s)  |
|--------------------------------|--|---|---|---|
| Alanine aminotransferase (ALT) | IFCC reference measurement procedure (37 °C) for ALT     | Lyophilised, fresh, or frozen human serum or plasma | Kinetic spectrophotometry                                 | Clin Chem Lab Med 2002;40:718-24                      |
| Creatine kinase (CK)           | IFCC reference measurement procedure (37 °C) for CK      | Lyophilised, fresh, or frozen human serum or plasma | Kinetic spectrophotometry                                 | Clin Chem Lab Med 2002;40:635-42,739-45               |
| Cholesterol                    | DGKC Definitive method for serum cholesterol             | Lyophilised, fresh, or frozen human serum or plasma | Isotope Dilution / Gas Chromatography / Mass Spectrometry | Siekman L et al. Z Anal Chem 1976;279:145-6           |
| 17-hydroxy-progesterone        | Mayo Clinic reference method for 17-hydroxy-progesterone | Human serum   | Liquid Chromatography / Tandem Mass Spectrometry          | Wudy S, Hartmann M, Svoboda M. Horm Res 2000;53:68-71 |



**Figure 1.** Traceability of measurement results according to EN ISO 17511 showing the reference materials and reference measurement procedures used to establish concentration or activity values for commercially available calibrator and quality control material used in routine methods for several analytes.

Laboratories listed in JCTLM provide the required link between routine laboratories and the reference materials and measurement procedures of higher metrological order.<sup>6</sup>

**Level 3: Routine (Testing) Laboratories**

These laboratories provide the routine measurement services to the medical community and must demonstrate their competence through participation in EQA Programs (e.g. Royal College of Pathologists of Australasia Chemical Pathology Quality Assurance Programs Pty. Ltd.) and accreditation (e.g. National Association of Testing Authorities, Australia). They are outside the recognition of the JCTLM and do not get listed.

**Split-Patient Sample Measurement in Traceability**

Traceable calibration does not necessarily produce traceability of test results. The requirements to establish traceability of test results depend on the following factors:

**1. Trueness value assignment:** The value transfer from certified reference materials to the manufacturer’s calibrators used in routine measurement systems follows a standard protocol. The main components in value transfer are the alternating reference measurement procedures and reference materials (Figure 1). Each link in the value transfer has its stated uncertainty under defined measurement conditions. The strict adherence to the value transfer protocol ensures that trueness is transferred successfully.

**2. The correct matrix of the calibrator:** The value assignment to higher order reference materials should be independent of matrix. Commutable, serum-based secondary reference materials ensure successful value transfer to the manufacturer’s calibrator. However, the manufacturers may use reduced serum or commercial matrix which might show different behaviour from that of patient samples under the same assay conditions (non-commutability).

### 3. Analytical specificity and sensitivity to interference of the comparison method:

It is critical that the chemical signal from the test sample used for comparison with the calibrator signal is only attributed to the analyte of interest. The test sample results will not be traceable if the method of comparison partially detects substances which do not form part of the measurand, e.g. cross-reactants or interferences. Non-specific methods, eg Jaffe procedures for creatinine, can never be traceable.

A split-patient sample comparison is used to compare two measurement procedures that operate at the same metrological level.<sup>9</sup> The manufacturer may use a panel of single donation patient samples with values assigned using primary reference materials and test them using the same measurement procedure calibrated with manufacturer's calibration materials. The results are compared using regression analysis and if necessary, correction factors will be used to compensate for any deviation shown by manufacturer's calibration materials due to any artificial matrix effect. Although this type of practice is common, it is considered non traceable under the rules of ISO 17511. The November 2007 issue of *The Clinical Biochemist Reviews* includes an excellent review on traceability in clinical enzymology<sup>10</sup> and is recommended for further reading in this area. A full review has been provided by Panteghini.<sup>11</sup>

### Conclusions

Introduction of traceable calibrators will support mobility of patients across geographic areas as inter-method and inter-laboratory results of measurement will be comparable. Laboratory results will form part of portable medical records allowing effective monitoring of patient treatment or disease. In addition, healthcare professionals in different geographic locations will benefit from international clinical studies. The use of a common reference interval will also be possible and the benefits from the contribution of laboratory results to the evidence based medicine literature will be significant.

On the other hand, results of measurements are only useful when compared against a previously established result of measurement, decision limit, reference interval and to a certain extent the experience of the practising physician. The introduction of traceable calibrators will inevitably change the quantities of measurement results and therefore the reference values will also change.

This may lead to temporary misinterpretations of the results until such time that the physicians are familiar with the change. The laboratory's role in communicating this potential effect on patient management should form a key activity of its routine functions.

Although traceability of results may appear to be a long way off, it is probably closer to realisation than we imagine. New legislation now mandates such a requirement. Data available on the BIPM website provide a significant step forward. As a starting point a laboratory should initiate communications with their respective suppliers of test kits and investigate the availability of traceability data for calibrators. Most manufacturers already have available traceability information that will allow the clinical laboratory and their clinicians to begin benefiting from traceability of measurement results.

**Competing Interests:** None declared.

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