# *Implementation of Reference Systems in Laboratory Medicine*

In laboratory medicine, meaningful measurements are essential for the diagnosis, risk assessment, treatment, and follow-up of patients; therefore, methods applied in diagnostic measurements must be accurate, precise, specific, and comparable among laboratories *(1)*. A given analytical measurement has only one true result, and the reliability of a measurement lies both in the result obtained and in the performance of a well-defined measurement procedure. Inadequate or incorrect analytical performance has consequences for the patient, the clinician, and the healthcare system. Poor-quality laboratory results may lead to incorrect interpretation by the physician, to a wrong diagnosis, and hence to treatment that impairs, or at least does not help, the patient's situation.

Measurement procedures in laboratory medicine should not be based on consensus but must follow the established rules of metrology as outlined in the *International Vocabulary of Basic and General Terms in Metrology* (VIM) *(2)*. According to VIM, metrology includes all aspects of measurements in whatever fields of science or technology they occur. One key element of metrology is the traceability of a test result to the International System (SI), which ensures comparable results for different measurements of the same analyte in the same sample. "Traceability" is defined as the property of the result related to national or international standards through an unbroken chain of comparisons, each of which has stated uncertainties *(2)*. The importance of these metrologic principles is described in an International Organization for Standardization (ISO)/European Committee for Standardization (CEN) standard *(3)*. These rules must be followed if results of diagnostic measurements are to be comparable and true wherever in the world they are performed. Given the increased mobility of patients, comparable (true) test results are essential for a rational and cost-effective diagnostic approach. To reach these goals, international and regional organizations such as the ISO, the CEN, the IFCC, the International Union for Pure and Applied Chemistry (IUPAC), the International Council for Standardization in Hematology, and the NCCLS have agreed on a metrologically sound Reference System.

In laboratory medicine, a Reference System consists of a network of reference laboratories that use Reference Methods and Certified Reference Materials (CRMs) for optimal measurement of analytes in various biological matrices *(4–8)*. Reference laboratories, analytical centers of competence, perform measurements with the greatest competence and are considered expert institutions for quantifying certain well-defined analytes, using the best, internationally agreed-on measurement procedure. Their main responsibility is to assign target values to Reference Materials, using the best analytical method available. In addition, they establish for so-called routine methods the extent of associated analytical bias in comparison with primary methods, with established Reference Methods, or if no Reference Method has been developed, with designated comparison methods. Thus, these laboratories establish through a chain of comparisons the traceability of routine methods and their respective biases.

According to the International Centre for Metrology, isotope dilution with mass spectrometry, coulometry, gravimetry, titrimetry, and determination of freezing-point depression are primary methods, yielding results in SI units (moles) without requiring reference to a standard *(9)*. However, these kinds of methods are applicable only for the measurement of elements and of exactly defined metabolites, which is not the case for many of the analytes used for clinical diagnosis. In laboratory medicine, the exact definition of the analyte, its biological and clinical function, and the influence of the matrix are crucial elements when establishing a Reference Method. An analyst first must know what to measure before deciding by which means the analyte (enzyme, protein, isoform, or metabolite) can be measured. These two steps usually are formalized by an expert committee. The Reference Method (the highest possible level in the metrologic hierarchy) needs to be specific for the defined analyte, and the chemical, biochemical, or immunological reaction used in the method must be well defined and completely described. Moreover, a statement of the uncertainty of the measurement must be included. These methods must be reproducible in time and space, which means that if the description of the method is followed, the true values obtained in a certain sample must be within the described uncertainty. When based on the most up-to-date knowledge, Reference Methods are considered "Definitive Methods". In cases where no Reference Method is available for an analyte, a so-called designated comparison method can be established according to the principles mentioned above. After extensive analytical investigations, such methods might evolve into Reference Methods. Accordingly, a designated comparison method can be a candidate for a Reference Method, but it should not be treated as an alternative to an existing Reference Method that is considered troublesome or too expensive *(10)*. The overall objective in the hierarchy of measurement procedures is to achieve the highest possible analytical quality.

Within this Reference System, biological CRMs play a key role, because the analyte concentration in a patient's sample is measured by comparing its signal with the signal given by the standard/calibrator. CRMs are either primary or secondary matrixed reference materials. CRMs carry a value for the analyte as measured with a defined uncertainty by a primary method or a Reference Method by reference laboratories. The starting points in the preparation of a CRM are the pure or purified analytes from human origin and the matrix. In the case of proteins, recombinant preparations for which the structure, amino acid composition, and degree of glycosylation have been established can also be used. The values are assigned to the pure analyte by Definitive Methods and transferred to

**Table 1. Criteria for international CRMs.**

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| Matrix | Similar to a patient specimen (serum, plasma, urine, cerebrospinal fluid) |
| Analyte(s) | Exact biological and structural identification |
| Analyte quantity | Certified value traceable to SI |
| | Uncertainty statement for the assigned value |
| Measurement results obtained | Commutable: constant numerical relationship with different measurement procedures for all kinds of clinical conditions |
| Purpose | Calibration of manufacturer's master calibrators |
| Availability | Worldwide |
| Stability | Over years |

Matrix Reference Materials by the use of Reference Methods. Values are assigned to CRMs by reference laboratories using standardized, well-defined conditions. Usually, the value for the primary Reference Material is used to calibrate the value transferred to the matrixed CRM. Use of poorly characterized methods with unknown trueness or of calibrators of lesser standardization is not acceptable for value assignment.

When such approaches are used for analytes measured by immunoassays, as often happens, the comparisons serve only to comfort those participating in the exercise, at least when their results agree with others. This kind of exercise, and the results obtained, may describe the reproducibility of quantitative methodological differences, but they are not accurate, not traceable, and not in agreement with the principles of metrology. When an immunoassay is used to certify the value of a Matrix Reference Material, exact definition and characterization of the analyte and the reagent (including the antibody) are essential for international acceptability of the values assigned.

Matrix properties similar to patients' specimens, commutability, and true and accurate assigned values are the essential criteria for biological CRMs. The criteria for international CRMs in laboratory medicine are summarized in Table 1 *(11)*. The main purpose of these international CRMs is generally considered to be value-transfer to the master calibrators used by manufacturers to calibrate their test systems. The procedure for certification of a secondary Reference Material follows international guidelines released by WHO, ISO, and the European Commission *(12–14)*. To convince manufacturers to accept the principles of metrology and to demonstrate improvement of the quality of test results in laboratory medicine by use of international CRMs, a feasibility study with expert laboratories demonstrating improvement of comparability, commutability, and the impact on standardization should be included in each certification process. In this context, the requirements of traceability of values assigned to CRMs and calibrators must again be stressed. This can be achieved only by use of internationally agreed on Reference Methods or the best method available, e.g., gas chromatography/isotope dilution/mass spectrometry. The concept of metrologic traceability and the hierarchy of analytical measurement procedures

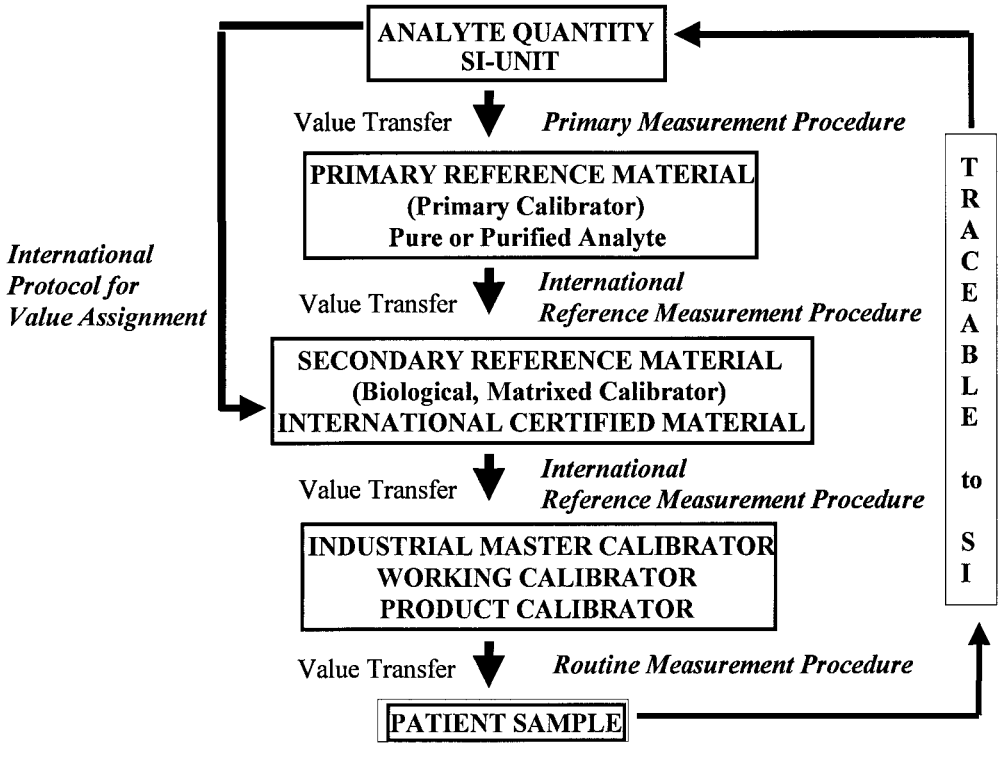


Fig. 1. Calibration hierarchy and traceability to the SI.

are shown in Fig. 1 and are based on a recent ISO/CEN standard for measurements of patients' samples *(3)*.

The recent European directive on in vitro diagnostics *(15)* follows this ISO/CEN standard and requests application of the standard for all in vitro diagnostic reagents used within the European Union. This new European legislation will have worldwide impact. A recent press release of the European Commission, "EU and USA to Measure Together", summarizes the arrangements, multilateral recognition, and ongoing activities for cooperation in metrology and measurement standards between NIST, in the United States, and the European Metrology Institutes. Thus the outlined Reference System, involving national metrology institutes, international scientific professional organizations, and collaborating reference laboratories, will be implemented.

Certainly the outlined principles of metrology will be applied in laboratory medicine as in other analytical disciplines; this is essential for measurement results that are comparable worldwide *(16)*. In addition to the rules of metrology, however, the clinical usefulness, the diagnostic needs, and the biological and disease-associated variations in analytes in patients' specimens must be taken into account when defining what analytical biases are acceptable for diagnostic purposes. Diagnostic laboratories must have the general goal of producing results that are true and comparable worldwide, which can be achieved by improving our metrologic consciousness. The contribution of our field in evidence-based medicine will then be easier to demonstrate.

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