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Reference Systems in Clinical Enzymology

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At the IFCC General Conference in Sevilla, Spain (March 1998) it was decided to establish a global reference system for the measurements of catalytic concentrations of enzymes comprising the following elements:

- Reference Measurement Procedures The existing 30 °C IFCC reference methods are used as a basis for developing a set of standard operating procedures (SOPs) for a reaction temperature of 37 °C
- Network of Reference Laboratories A group of reference laboratories (including manufacturers' laboratories) are selected to provide the necessary skill and equipment to carry out measurements following the reference measurement procedures (SOPs) on a high metrological level.
- Reference Materials The existing BCR reference materials are to be re-certified by the network reference laboratories according to the co-operation contract between IFCC and IRMM. For some enzymes (AST, Amylase, Lipase) it will be necessary to establish and to certify new commutable reference materials.

With the introduction of such reference systems we are following the concept of measurement traceability that has been established in general metrology and which is now also introduced to the field of clinical chemical analyses. Traceability probably provides the most important strategy to achieve standardisation in laboratory medicine aimed at comparable measurement results regardless of the method, the measurement procedure (test kit) and the laboratory where analyses are carried out.

Consequently, the In Vitro Diagnostica Directive [1] 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 metrological order.

Furthermore, the European Commission has mandated standards on the traceability of values assigned to calibrators and control materials in general laboratory medicine [2] as well as in the specific field of the measurement of catalytic concentrations of enzymes [3].

According to the Vocabulary in Metrology (VIM) [4] and the Guide to the Expression of Uncertainty in Metrology (GUM) [5], measurement traceability is defined as

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 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 an increasing hierarchical order as shown in Fig.1. 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 use of a primary reference procedure; this, of course, is not feasible.

An inevitable precondition for the establishing of traceable results to 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. As it concerns the measurement of the catalytic activity concentration of enzymes it is necessary that the routine and lower order procedures exhibit similar selectivities with respect to the individual isoenzymes and molecular forms.

The complete traceability chain is valid only for those measurable quantities that 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. The question arises whether results of measurements of catalytic concentrations of enzymes can be traceable to the SI unit or only to a lower level in the hierarchical traceability chain.

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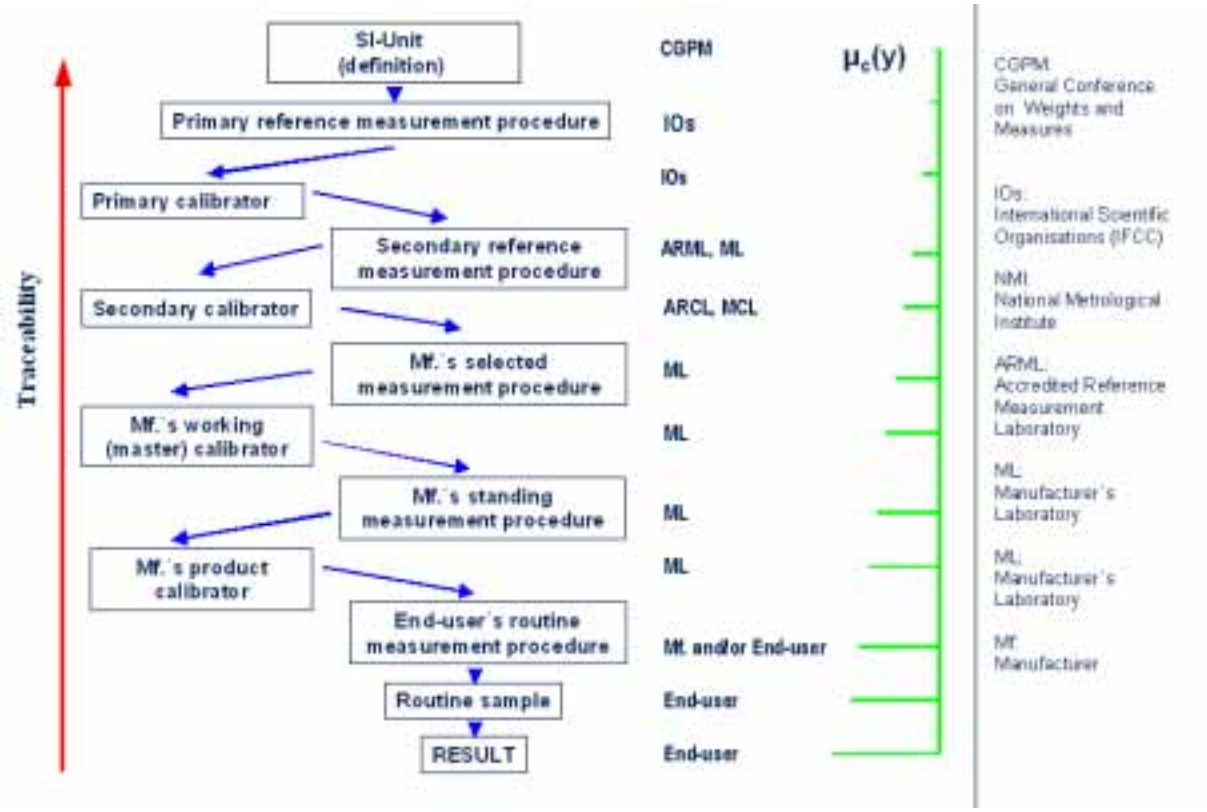

Figure 1

At first glance, the situation seems to be more difficult for enzymes than for other quantities such as electrolytes or metabolites, e.g. cholesterol, which obviously can be described by the SI unit 'mol per litre'. In contrast, results of catalytic concentrations of enzymes are only comparable when the enzyme activities are measured under the same conditions.

Therefore, an enzyme measurand cannot be described only by its name, kind of quantity and system, e.g. 'catalytic activity concentration of creatine kinase in human serum', but its definition requires also the specific measurement procedure. Although a short name for the enzyme analyte is generally used in clinical enzymology, e.g. 'creatine kinase', the measurand is in fact defined as "catalytic activity of the enzyme as measured by the conversion rate of an indicator substance in a specified system according to a given measurement procedure" e.g. "catalytic activity of creatine kinase as measured by the rate of conversion of NADH in the IFCC reference procedure".

In principle, this procedure-dependence is not unique for the measurement of catalytic concentration of enzymes, but applies also for much simpler quantities. For example, the measurement of the length of a rod of iron requires the description of measurement conditions, at least of the actual temperature, or, the measurement of total cholesterol in serum requires a statement on the step of hydrolysis.

In the hierarchical system of reference procedures and materials the IFCC enzyme reference measurement procedures shall form the highest metrological level and thereby constitute the definitions of the respective measurable quantities. According to international legislation (EU-IVD directive) [1] and international standards (ISO, CEN) [2, 3], values assigned to calibrators and measurement results of lower metrological level, including those used in daily routine practice, should be traceable to top level reference measurement procedures and the SI unit (Fig. 1).

The coherent derived SI unit of measurement "mole per second cubic metre", symbolized as: mol s-1 m-3 [or (mol/s)/m3], also called 'katal per cubic metre', symbolized as: kat m-3 (or kat/m3), shall be the top of any calibration hierarchy for catalytic concentration of an enzyme [3].

According to the standard 'Metrological Traceability of Values for Catalytic Concentration of Enzymes Assigned to Calibrators and Control Materials' [3] the enzyme measurands are defined by the primary reference procedures which have to be followed in all details, e.g. - kind of substrate and its concentration, - buffer components, - pH value, - effectors and their concentrations, - direction of the catalysed reaction, - indicator components, - temperature, - incubation time, - lag-phase time, - measurement time, - wavelength.

Table 1: Current members of the Enzyme Reference Laboratory Network

Dr. F. Ceriotti	Istituto Scientifico San Raffaele	Milano	Italy
Dr. G. Ehlers	Ortho - Clinical Diagnostics	Rochester	U.S.A.
Prof. G.Ferard/ Dr. Lessinger	Centre Traumatologie et Orthopedie	Illkirch Grafenstaden	France
Dr. F. H. Franck	Ziekenhuis Leyenburg	Den Haag	The Netherlands
Prof. J. Gella	Biosystems S.A.	Barcelone	Spain
Prof. W. Hölzel	Roche - Boehringer Mannheim GmbH	Tutzing	Germany
Dr. P. Joergensen	Dept. of Clinical Chemistry	Odense	Denmark
Prof. T. Kanno	Hamamatsu University Hospital	Hamamatsu	Japan
Dr. A. Kessner	Beckman -Coulter, Inc.	Brea, CA	U.S.A.
Dr. M. Panteghini	1 Laboratory of Clinical Chemistry	Brescia	Italy
Dr. F. Schiele	Centre du Medecine Preventive	Nancy	France
PD Dr. G. Schumann	Med. Hochschule Hannover	Hannover	Germany
Dr. A. Vialle	Hopital Debrousse	Lyon	France
Dr. G. Weidemann	Klinikum der Stadt Nürnberg	Nürnberg	Germany
Dr. K.Yoshinari	ASAHI Chemical Industry Co.,Ltd.	Tokyo	Japan

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

The new 37°C IFCC procedures are based on the existing 30°C IFCC recommended methods [6, 7, 8, 9, 10, 11]. The measurement conditions were further optimised concerning substrate concentration, pH, buffer concentration, lag phase, and measurement time interval. This was necessary at least to some extent due to the change of temperature. The measurement conditions are described in the form of standard operating procedures in every detail.

Primary IFCC reference measurement procedures for ALT, AST, Amylase, CK, GGT and LD are currently prepared for publication. The development of procedures for AP, Lipase and CHE is projected.

The measurement procedures have been established in a network group of laboratories. Current members of the network are listed in Table 1

Each laboratory of the network agreed to follow stringent metrological principles:

Calibrated test weights were used for all gravimetric steps; for measuring and dispensing volumes, equipment was used that had been calibrated by gravimetry; for temperature and pH adjustments, calibrated devices with known uncertainties were applied. For testing the wavelength adjustment and photometric absorbance of the photometric equipment, filters and/or test solutions certified by a national metrology institute were applied.

All the relevant data contributing to the overall-uncertainty of the final results had to be reported to the co-ordinator of the network.

Finally, the group of expert laboratories (Table 1) certified, in collaboration with the Institute of Reference Materials and Methods of the European Union (IRMM), enzyme preparations which had been previously certified by different measurement procedures, generally at 30°C.

For the BCR ALT reference material, the certification campaign resulted in a very small 95% confidence interval, which was in the order of +/- 1% in a group of 12 laboratories. This small interval proved to be particularly satisfactory when compared to former certification campaigns using the same material and also to some training experiments with commercial control materials prior to the certification study (Fig. 2).

This was equally true for the certification of the LD reference preparation and for the BCR reference material for CK.

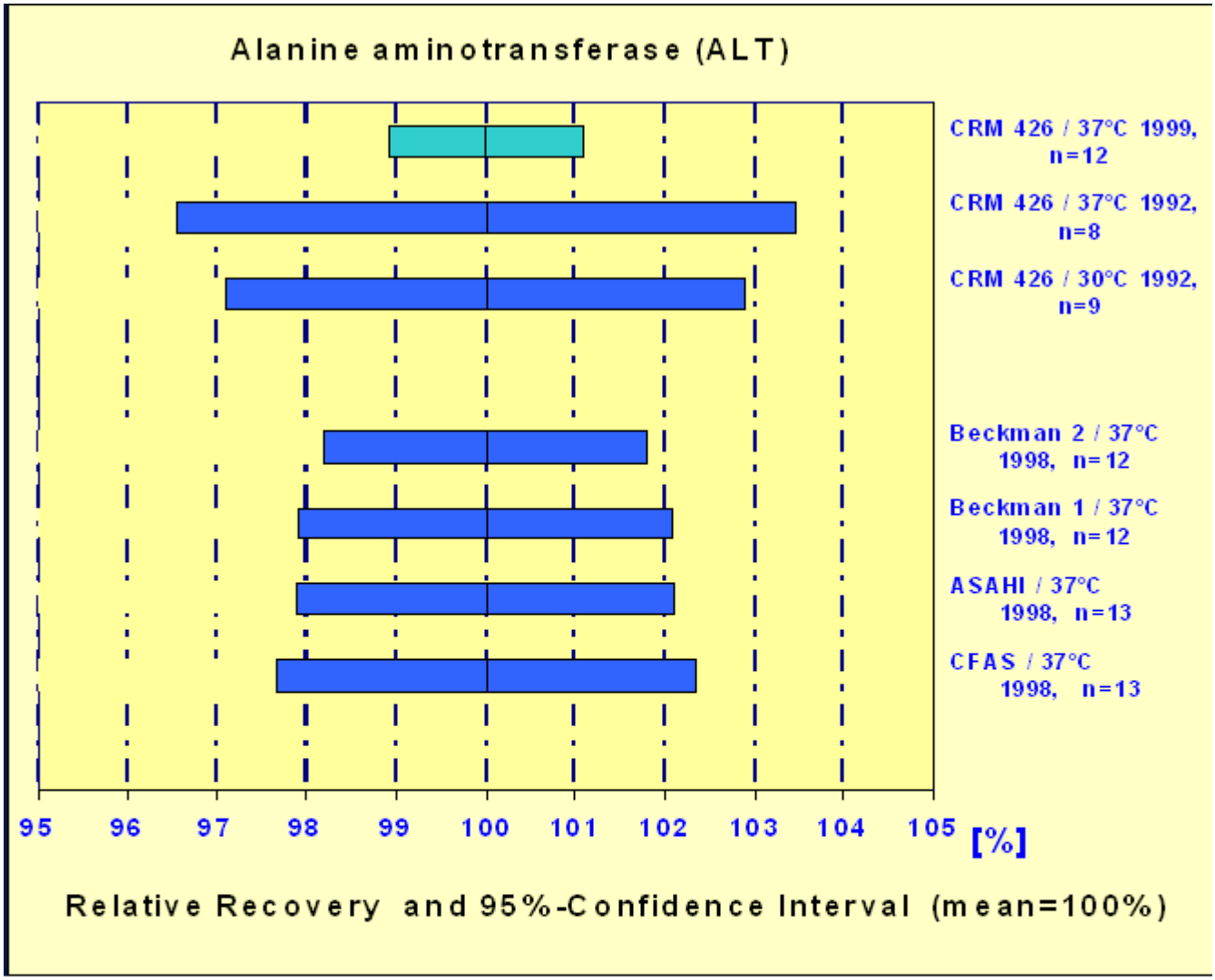

Fig. 2
Certification of IRMM-BCR reference material CRM 426 and various control materials for ALT.
95%-confidence-intervals obtained by different laboratories in the certification campaigns.
Top bar: Certification of CRM 426 by the new 37°C IFCC reference procedure (12 labs).
Second bar: Certification of CRM 426 by the former IFCC reference procedure at 37°C (8 labs).
Third bar: Certification of CRM 426 by the former IFCC reference procedure at 30°C (9 labs).
Fourth – seventh bar: Measurements of various control materials by the new 37°C IFCC reference procedure (12 labs) in a training experiment for the certification of the BCR reference material.

Also, for the certification of the BCR GGT reference material the scatter of results of the 12 laboratories in terms of the 95% confidence interval was below +/- 1.5%.

It should be mentioned that most recently also the BCR Amylase reference material has been certified with similarly good results.

This very satisfactory agreement of results obtained from different reference laboratories demonstrates

- first the good performance of the network laboratories from the far East (Japan) to the far West (California) including laboratories from universities, hospitals and manufacturers
- and, second, the high level of improvement and the exact description of the measurement procedures which were achieved thanks to the contributions of members of the network of reference laboratories.

As soon as the primary IFCC reference procedures are published after a mail ballot among the IFCC members (hopefully within the next few months), the complete reference system comprising reference procedures, laboratories and material, will be available. Then, the traceability requirement, as

formulated by the IVD directive of the European Union and in the CEN/ISO standards, has to be implemented not only by manufacturers when designing commercial test kits and calibrators for the measurements of catalytic concentrations of enzymes, but also by the organisers of external quality assessment schemes when assigning target values for their control materials, which are distributed to the participating laboratories.

In summary, it can be stated that a reference system has been established for the measurement of catalytic concentrations of enzymes, which may also serve as a model for other groups of quantities to be standardised in terms of traceability.

References

1 Directive 98/79/EC of the European Parliament and the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices.

2 prEN ISO 17511, In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Metrological traceability of values assigned to calibrators and control materials.

3 prEN ISO 18153, In vitro diagnostic medical devices - Measurement of quantities in samples of biological origin - Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials.

4 International vocabulary of basic and general terms in metrology, 2nd edition, ISO, Geneva, 1993

5 Guide to the expression of uncertainty in measurement, 1st edition, ISO, Geneva, 1995

6 Bergmeyer HU, Hørder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1). J Clin Chem Clin Biochem 1986;24: 497-510.

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