IFCC Committee on Reference Systems for Enzymes (C-RSE)

Status report on the activities in 2001

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NEW IFCC REFERENCE PROCEDURES FOR ALT, AST, CK, GGT and LD

A mail ballot was launched by the IFCC Board and Scientific Division (SD) to the IFCC member Societies. 31 IFCC members replied (all with YES). Although a simple majority is sufficient, the situation remained unsatisfactory as some votes of important countries, e.g. Canada, Australia, and the Scandinavian countries, were still missing. It was proposed to extend the mail ballot until February 28, 2002 and to further contact the national representatives of these countries to obtain their votes.

The seven documents, comprising the concept (introduction), the reference procedures for ALT, AST, CK, GGT and LD as well as the certification report of reference materials from the Institute for Reference Materials and Methods (IRMM), will be submitted to *Clinical Chemistry and Laboratory Medicine* for publication.

REFERENCE INTERVALS FOR ALT, AST, CK, GGT AND LD

Schumann's laboratory carried out a comprehensive study for establishing reference intervals for the above-mentioned five enzyme activities. 200 to 400 samples for each enzyme were selected from hospital patients without distinctive features in a variety of routine clinical chemical tests for metabolic disorders. Contemporaneously, the two italian laboratories of the IFCC network (Brescia and H. San Raffaele Milano) reported on a study for establishing reference intervals for ALT, AST and LD, using samples from healthy blood donors. The selection of individuals was done using the IFCC recommendations for the production of reference values (1).

The results of the two studies were compared with the data given as preliminary tentative upper reference limits in the documents which will be submitted for publication as primary IFCC reference procedures. These preliminary reference limits were then modified accordingly as follows:

- ALT females: ≤ 34 U/L, males: ≤ 45 U/L
- AST females: ≤ 31 U/L, males: ≤ 35 U/L
- CK females: ≤ 145 U/L, males: ≤ 171 U/L
- GGT females: ≤ 38 U/L, males: ≤ 55 U/L
- LD all: ≤ 247 U/L

In particular, it was decided to publish the above-mentioned upper reference limits as *preliminary data,* which should not prevent in future more extensive studies.

PROVISION AND COMMUTABILITY OF THE AST REFERENCE MATERIAL

A recombinant human AST material (cytosolic isoenzyme), prepared by the Asahi Kasei Co., was selected as a candidate reference preparation. Two laboratories from the IFCC network will preliminarily check the commutability of a pilot batch of this material. Samples from patients and from the pilot batch preparation will be measured in parallel by the IFCC AST reference procedure and a routine test system in each of the two laboratories. Contemporaneously, the process of lyophilisation for preparing the actual Asahi material will be evaluated at the IRMM. For the material certification, further network laboratories will be involved.

STATUS OF THE 37°C IFCC REFERENCE PROCEDURE FOR AMYLASE

The amylase procedure, originally published by Lorentz, was optimised for the measurement at 37°C by the C-RSE and used for the certification of the IRMM 476 reference material (2,3).

After certification of this reference preparation, demonstration of the commutability of this material is required. The study will be carried out by some laboratories of the IFCC network with a series of patient samples in a relevant activity concentration range and the IRMM reference material using the IFCC reference procedure and various routine procedures in parallel. The participating reference network laboratories will apply the following amylase routine test kits: G7-substrate (Beckman Coulter and Roche Diagnostics), G3-substrate (BioMerieux, BioSystems and Merck).

The standard operating procedure for amylase will be submitted to the IFCC SD for a mail ballot distribution to the IFCC members. In the meantime, Junge et al. has collected data on the reference interval which will be published soon (4).

OFFICIAL APPROVAL OF THE NETWORK OF REFERENCE LABORATORIES

Three meetings of representatives of the IFCC, the IRMM, the International Committee for Weights and Measures (CIPM), the Consultative Committee on Amount of Substance (CCQM) and national metrology institutes were held during 2001. A 'Joint Committee on Traceability in Laboratory Medicine' (JCTLM) was established with the aim to support world-wide traceability, comparability, and equivalence of measurement results in Laboratory Medicine (Table 1) (5).

The national metrology institutes will be responsible in principle for the traceability of any measurement in all countries which signed the 'meter convention'. In cases where the national metrology institutes do not feel competent, e.g. in enzymology, they should delegate their competence to expert laboratories in their countries. The metrology institutes will demonstrate their competence and that of reference laboratories by so-called "key comparisons" which are ring trials, i.e. regular independent measurement comparisons in a network, of these laboratories. In Laboratory Medicine, such key comparisons has already been conducted for cholesterol, creatinine and glucose in human serum using procedures of a high metrological level, e.g. isotope dilution mass spectrometry. Under the umbrella of this infrastructure as provided by the metrology institutes, reference laboratories may serve scientific community, manufacturers, organizers of External Quality Assessment Schemes (EQAS) and other interested parties. In such a system, the laboratories may receive accredited status of 'reference laboratories' according to ISO 17025 and/or ISO 15195 documents (6).

As a basis for such an acknowledgement, the IFCC should express an approval of such network laboratories in a particular field of interest on the basis of regular inter-laboratory comparisons. Different concepts to establish rules for such a network have been discussed. An approval of the IFCC could be expressed on the basis of an outcome of a laboratory

Table 1
Objectives of the Joint Committee on Traceability in Laboratory Medicine (JCTLM)

- Promoting the concept of traceability of measurement results to the SI or to other internationally agreed references where traceability to the SI is not yet feasible;
- In cases where traceability to the SI is not yet feasible (e.g. measurement of enzyme catalytic activity), co-ordinating and giving guidance in the establishment of a Reference Measurement System with respect to medical needs where such an international conventional system is applicable;
- Identifying and prioritizing the measurands requiring international traceability and comparability and encouraging appropriate organizations to accept responsibility for the development of suitable reference measurement procedures and certified reference materials;
- Disseminating relevant information to all interested parties;
- Providing scientific and organizational expertise to the parties involved;
- Encouraging the application of reference systems by the In Vitro Diagnostics industry.

inter-comparison for each individual enzyme and the reported data on all calibration tests of the laboratory equipments; alternatively, an approval could be expressed for all enzyme that are measured, for example, by using the same analytical principle, e.g. photometric absorbance, on the basis of the measurement comparison for only one selected enzyme. The frequency of comparative measurements could be once or twice a year. The IRMM would support the infrastructure, by conducting certification campaigns, commutability and stability studies, which could also serve as inter-laboratory comparison experiments.

DEVELOPMENT OF A REFERENCE PROCEDURE FOR ALKALINE PHOSPHATASE

Ceriotti's and Panteghini's laboratories on behalf of the SIBioC Working Group on 'Enzymes' performed a study comparing the two different buffer systems [amino-methyl-propanol (AMP) and N-methyl-glucamine (MEG)], previously recommended for alkaline phosphatase (ALP) reference procedures (7,8).

Patient samples and a series of control materials were analysed by modifying various measurement conditions in order to check the robustness of the system in view of the commutability of the control materials. It turned out that, no matter which of the two buffer systems was used, the values for most of the control materials changed dramatically when the measurement conditions were modified, whereas the effects were less pronounced when patient samples were evaluated. Obviously, the majority of the currently available control materials are not commutable since they consist of isoenzymes different from the pattern which is usually detected in cases of liver or bone diseases. In particular, the use of placental ALP to spike control materials should be discontinued by the manufacturers. It would be desirable to use recombinant tissue non-specific ALP to prepare control materials. Asahi representatives announced that they are able to offer a new recombinant tissue non-specific ALP material. A similar recombinant material will also become available from Roche Diagnostics. The commutability studies will be extended using such recombinant human tissue non-specific ALP preparations, either in AMP or MEG buffer test systems.

DEVELOPMENT OF A REFERENCE PROCEDURE FOR LIPASE

In November 2001, Lessinger and Ferard submitted a proposal to IFCC SD for a lipase reference procedure based on titrimetric method. With regard to this enzyme, some problems may arise from the fact that most of the commercially available routine procedures for lipase actually measure different esterases, whereas the proposed reference procedure specifically determines lipase; in so far different analytes can be measured by the reference procedure and the routine test kits.

A group of five independent laboratories has already used the procedure to certify a candidate reference material (lipase from pancreatic juice in human albumin). Unfortunately, no details on the comparative measurement campaigns are available. Also, data for the commutability of this candidate material using a sufficient number of patient samples in a relevant measurement interval should be reported on.

DEVELOPMENT OF A REFERENCE PROCEDURE FOR SERUM CHOLINESTERASE

The SIBioC Working Group on 'Enzymes' has recently performed a pilot study to identify an optimal substrate for measurement of cholinesterase catalytic activity in serum (9). It was found that the use of succinyldithiocholine was of advantage when the cholinesterase test is used as a screening procedure in advance to anaesthesia for the detection of atypical genotype (and phenotype) patterns.

In Germany and in Japan, cholinesterase is rarely used as screening procedure in anaesthesia but as a marker for reduced protein biosynthesis in the liver and to detect poisoning by organophosphate and carbamate esters. In view of this application, a national German reference procedure has been developed based on butyrylthiocholine

as substrate (10). However, succinyldithiocholine substrate is equally suitable for both applications, so that a common reference procedure can be developed (11).

DEVELOPMENT OF A REFERENCE PROCEDURE FOR GLUTAMATE DEHYDROGENASE

This is obviously a typical German test which is rarely used in other countries (e.g. in some Italian hospitals). For this reason, there seems to be no common interest to develop an international reference system for this enzyme.

PROJECT 'CALIBRATION 2000'

In the Dutch national 'Calibration 2000' project, a candidate secondary reference material for the enzymes has recently been developed, using human serum spiked with human enzymes produced by recombinant DNA techniques, including ALT, AST, CK, LD, ALP, GGT and amylase. It was proposed to extend the Dutch project into an *International Pilot Project 'Calibration 2000 Enzymes'* for the development of an international secondary 'matrix'-based reference material, on the basis of the Dutch material for the enzymes. This is intended for use within Enzyme Reference Systems as described in ref. 5. Currently, some commutability data based on comparisons of different routine procedures are available. Experiments using the IFCC 37°C reference procedures are however lacking. It will therefore be necessary to demonstrate the commutability of the material by using the IFCC reference procedures in parallel to various routine test kits. It would also be valuable to demonstrate the usefulness of this material in a national EQAS.

REFERENCES

1. PetitClerc C, Solberg HE. IFCC approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. Clin Chim Acta 1987; 170:S3-12.
2. Lorentz K. Approved recommendation on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 9. IFCC method for α-amylase. Clin Chem Lab Med 1998;36:185-203.
3. Panteghini M. IFCC Committee on Calibrators in Clinical Enzymology (C-CCE) - Status report on the activities in 2000. Biochim Clin 2001;25:273-4.
4. Junge W, Wortmann W, Wilke B, Waldenstrom J, et al. Development and evaluation of assays for the determination of total and pancreatic amylase at 37°C according to the principle recommended by the IFCC. Clin Biochem 2001;34:607-15.
5. Panteghini M, Ceriotti F, Schumann G, Siekmann L. Establishing a reference system in clinical enzymology. Clin Chem Lab Med 2001;39:795-800.
6. International Organization for Standardization. Laboratory Medicine - Requirements for reference measurement laboratories in Laboratory Medicine. ISO/TC 212/SC N78 ISO/FDIS 15195. Geneva, Switzerland: International Organization for Standardization, 2001.
7. Tietz NW, Rinker AD, Shaw LM. IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase. J Clin Chem Clin Biochem 1983; 21:731-48.
8. German Society for Clinical Chemistry, Working Group on Enzymes. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasma at 37°C. I. Alkaline phosphatase (orthophosphoric-monoester phosphohydorlase, alkaline optimum, EC 3.1.3.1). Eur J Clin Chem Clin Biochem 1992;30:247-56.
9. Mosca A, Patrosso C, Bonora R, Ceriotti F, et al. Genetic defects of serum cholinesterase: enzymatic activity, dibucaine and fluoride numbers, and genotype. Clin Chem 2000; 46(suppl): A174.
10. German Society for Clinical Chemistry, Working Group on Enzymes. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasma at 37°C. II. Cholinesterase (acylcholine acylhydrolase, EC 3.1.1.8). Eur J Clin Chem Clin Biochem, 1992;30:163-70.
11. Panteghini M, Bonora R, Pagani F. An alternative approach to the prevention of succinyldicholine-induced apnoea. J Clin Chem Clin Biochem 1988;26:85-90.