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Verslagen

Commercially available enzyme calibrators, an overview

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In order to reduce interlaboratory variation of enzyme results in clinical chemistry there is a definite need for commutable multienzyme calibrators with target values traceable to IFCC recommended methods. We have performed a market survey to collect information on commercial calibration sera that are currently available. We have requested specific information on the stability, the exact contents, the source of the added enzymes, the commutability and the procedures used to assign target values or ranges.
No calibrator exists today, that fulfils all criteria. Four calibration sera may have potential as calibrator for enzyme assays. However, two lack information on commutability, two lack information on traceability and one is still in an experimental phase. Finally, the traceability of the calibration sera is discussed and also the commutability in relation to the contents of the calibration sera.

Key-words: enzyme calibrator; reference method; quality assessment; IFCC; DGKC

Despite many efforts to standardize enzyme activity measurements in clinical chemistry, interlaboratory variance of enzyme activity still needs much improvement. Method standardisation alone was not found to be sufficient to achieve satisfactory interlaboratory variation and the need for calibration to overcome the remaining interlaboratory variation is obvious. This viewpoint has been adopted and is propagated by joint efforts of the CEN/TC140 (Technical Committee 140 of the European Committee for Standardisation) and the IFCC working group on Calibrators in Clinical Enzymology. Already in 1983 experiments using control sera have shown the feasibility of such an approach (1). Recently, the Dutch foundation for quality assessment in clinical laboratories (SKZL) has started the ”calibration 2000” project in order to achieve national harmonisation of clinical chemical results. This has resulted in a position paper, suggesting a model for the national harmonisation of enzyme results (2).

Using enzyme calibrators, calculation of enzyme activities is no longer directly based on the molar absorptivity of the substrate or reaction product but on assigned values of the calibrators. Such enzyme calibrators should meet several specifications (3-7):

- The enzyme calibrator should be commutable with fresh human sera for the various methods being used. Commutability of enzymes has been defined by “the ability of an enzyme material to show interassay activity changes comparable to those of the same enzyme in human serum” (8). To obtain commutability the catalytic properties should be as equal as possible to those of the enzymes in patient samples. Therefore, information on the characteristics of the enzymes used in the calibrator (Michaelis Menten constant (Km), maximal rate of substrate conversion (Vmax), inhibition constant (Ki), isoenzyme constitution, etc.) should be well documented like in Gruber et al. 1977 (9). In some sera stabilizers are added, such as ethylene glycol or sucrose, to obtain reproducible reconstitution after lyophilisation. Furthermore, cofactors like zinc ions or pyridoxal phosphate may be added. These variables may all affect the commutability of these materials and should therefore be documented.
- The enzyme calibrator should have assigned values obtained by measuring the enzyme activity either directly with a reference method using the molar absorptivity, or with a derived method that is directly traceable to the corresponding reference method. Therefore, it is necessary to know exactly the methods used to establish the enzyme concentration of the calibrator.
- The enzyme calibrator should be stable over a pro-

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Footnote:
Abbreviations: ALAT, alanine aminotransferase (EC 2.6.1.2); AP, alkaline phosphatase (EC 3.1.3.1);AMYL, alpha-amylase (EC 3.2.1.1); ASAT, aspartate aminotransferase (EC2.6.1.1); BCR, Community Bureau of Reference; CK, creatine kinase (EC 2.7.3.2); CRM, certified reference material; DGKC, Deutsche Gesellschaft für Klinische Chemie; γ-GT, gamma-glutamyltransferase (EC 2.3.2.2); IFCC, International Federation for Clinical Chemistry; JSCC, Japanese Society for Clinical Chemistry; LD, lactate dehydrogenase (EC 1.1.1.27); NIST, National Institute for Standards and Technology, USA; SFBC, Societé Française de Biology Chimique; SKZL, Dutch foundation for quality assessment in clinical laboratories; SSCC, Scandinavian Society for Clinical Chemistry.

longed period and it has to guarantee reproducibility upon reconstitution of lyophilized materials, without prolonged reactivation procedures.

Enzyme calibrators fulfilling these requirements can be used to assess long term accuracy and to reduce interlaboratory variations (5,10). Information on the specifications mentioned above is generally not documented in the package insert of the calibration sera. Therefore, information was collected from various manufacturers of enzyme calibrators by personal communication. These data are summarized and discussed in this review.

Outcome of the market survey

Only four enzyme calibration sera with applicability beyond the limitation of a single analyzer were found (table 1). All preparations are provided with information about the stability after reconstitution. All lyophilized products can be reconstituted with redistilled water or specific reconstitution fluid within 30 minutes by gentle stirring or standing. In contrast with earlier control sera, reactivation of AP is not required for any of the materials. None of the materials was provided with information on source and kinetic characteristics of the added enzymes and commutability. This information was received upon request.

Table 1. Commercial available enzyme calibrators and the origin of the added enzymes(species and organ)

Enzyme Calibrator	BCR-CRM Institute for Reference materials and measurements	Enzyme Verifier Bio-Rad Lab. Veenendaal The Netherlands	Seraclear-HE Enzyme reference WAKO Pure Chemicals Sopachem The Netherlands	Calibration serum Randox Lab. LTD Sanbio BV Uden The Netherlands	Roche Cfas Diag. BV Almere The Netherlands
Cons*titution Status*	lyophilized	liquid	lyophilized	lyophilized	lyophilized
Additives		confidential Zn^{2+}, Pyr5'-P preservatives	HEPES Sucrose Pyridoxal 5'P Ca^{2+}, Mg^{2+}, Zn^{2+} Folic acid	none	no information
CK	Human hart (MB, CRM 608) placenta (BB, CRM 299)	Human bone/muscle gene	Human embryo kidney cell line (EK)	Porcine hart	Rabbit muscle
LD	Human isoenzyme 1 (CRM 404)	Human erythrocytes	Human erythrocytes	Bovine heart	Porcine heart
ASAT	n.a.	Human erythrocytes	Human erythrocytes	Porcine heart	Porcine heart
ALAT	Porcine heart (CRM 426)	Human liver gene	Human hepatoma cell line (KN)	Porcine heart	Porcine heart
AP	Porcine kidney (CRM371)	Human liver gene	Human amnion cell line (FL)	Bovine mucosa	Human placenta
γ-GT	Porcine kidney (CRM319)	Human liver gene	Human macrophage cell line (M)	Bovine kidney	Porcine kidney
AMYL	Human pancreas (CRM476)	Human urine	Human urine(p-AMYL) saliva(s-AMYL) (n.a. in Enzyme Reference)	Porcine pancreas	Porcine pancreas

n.a.: not available

CFAS

Cfas (Calibrator for automated systems, Roche Diagnostics) consists of pooled human sera. The use of Cfas is intended to obtain comparable results between laboratories using the same method (= reagents from Roche Diagnostics) in combination with the corresponding target value of Cfas. Enzyme activity values are assigned to a masterlot of Cfas by measuring the enzyme activities at 37°C (and in some cases 30 or 25°C), using automated methods closely derived from the various (manual) reference methods. These 'automated reference methods' produce the same results in human sera as their manual counterparts (personal communication with Roche Diagnostics). Cfas is measured in duplicate in 6 independent laboratories on 3 successive days with the various routine methods calibrated with the Cfas master lot, and the mean results (target values) are given for each method. Subsequently, routine methods calibrated with commercial lots of Cfas are compared with the manual reference methods using deep frozen human serumpools. Thus traceability and long term stability are monitored. The assigned values of Cfas are determined in accordance with the guidelines of the Federal German Association of Physicians (Bundesärztekammer) and the European Committee for Clinical Laboratory Standards (ECCLS) (6). Target values are given for each method and temperature. The kinetic properties of the animal enzymes ASAT, ALAT, LD, CK and γ-GT in Cfas have been reported to be similar to those of the corresponding enzymes in fresh human serum under the experimental conditions commonly used in the period 1970 - 1980 (9,11). No information was available on the properties of the human placental AP and porcine pancreas AMYL. With respect to the commutability of Cfas with human serum enzymes, Roche refers to the same publications (9,11).

Calibration serum

Randox Laboratories provides a human serum based Calibration Serum having both method-, and instrument-dependent target values for a wide range of instruments, methods and temperatures. Target values are obtained from the consensus values of at least 10 independent laboratories for each instrument for each method. The participating laboratories use their analysers according to the instructions of the manufacturer and have not introduced any instrument factors. Furthermore Randox states that: "The mean of all instrument values produce identical results to the manual reference methods".

The kinetic properties of the added enzymes have been studied by Randox and found to be similar to those of human enzymes and preliminary comparison with an experimental all-human enzyme serum gave good commutability (personal communication with Randox).

Seraclear HE

Although WAKO Pure Chemicals has launched an enzyme calibrator (Seraclear HE) consisting of pooled human serum spiked with purified human enzymes partly derived from cell cultures, it is currently not available in Europe (12). WAKO also manufactures Enzyme Reference, containing the same purified human enzymes in a bovine albumin matrix. Enzyme Reference is available on request in Europe since the beginning of 1998 and is sold as Enzyme Calibrator. Target values were assigned using both JSCC and IFCC recommended methods (SFBC recommended method for LD). The kinetic properties of the added enzymes (12) and commutability for γ-GT, ASAT and ALAT (13,14) have been thoroughly investigated and were found highly comparable to human serum enzymes. Furthermore, significant differences in Km values between human enzymes and enzymes from various species were reported in these publications.

Enzyme Verifier

The applicability of a new Enzyme Verifier, consisting of human pooled serum spiked with prestabilised recombinant human enzymes derived from tissue culture or native enzymes from human erythrocytes or urine (manufactured by Asahi Chemical Industry Co., Tokyo, Japan), is presently being tested by Bio-Rad Laboratories. Commutability has been investigated by distribution together with a set of human poolsera to a group of laboratories in the region of Rotterdam, the Netherlands (15) (technical note in preparation, B.E.P.B. Ballieux). No target values have been assigned to the material yet. This year a new batch of the Enzyme Verifier will be tested by the SKZL for use in the calibration 2000 project.

CRM

Besides these multienzyme calibration sera, seven CRM preparations of the BCR are available for determination of the accuracy of enzyme methods (16-20). CRMs are preparations of highly purified animal enzymes in a bovine albumin matrix and information on the commutability is not available, with the exception of CRM 319 (γ-GT) (13). No CRM is available for ASAT. The fact that for each enzyme a separate CRM is needed makes them less suitable for routine use.

The IFCC working group on calibrators in clinical enzymology is now establishing new target values at 37°C for the CRMs. Results presented in a workshop at the IFCC/Worldlab 99 Conference in Florence (1999) show, that using the CRMs interlaboratory variation of the recommended methods between the members of the network of less than 2 percent is achievable.

Both Randox Laboratories and Bayer Diagnostics claim to be working on enzyme calibrators consisting of pooled human serum spiked with purified human enzymes.

Commutability of calibrators with human serum enzymes

Since the contents of the four investigated calibrators differ very much (table 1), commutability, if not documented by the manufacturer, is difficult to predict. Several aspects of commutability have been documented in literature.

For γ-GT, Cfas has commutability patterns closely resembling those of CRM 319 of the BCR, which has been prepared in cooperation with the Roche reference laboratory in Penzberg, Germany. Both Cfas and CRM319 contain porcine kidney γ-GT (13,16,17). Randox Calibration Serum contains bovine kidney γ-GT. Commutability of porcine- and bovine kidney γ-GT has been described to be limited, since calibrators containing porcine γ-GT could only be used to reduce intermethod variability between methods using L- γ-glutamyl-4-nitroanilide or the 3-carboxy-analog (recommended methods of the IFCC, DGKC and others). Calibrators containing bovine were not effective at all in reducing variability. This limited commutability or lack of commutability is indeed reflected by differences in the Km value between the nonhuman enzymes and human γ-GT (13,21). The commutability of ASAT isolated from porcine heart (Cfas and Enzyme Calibrator from Randox) is, like porcine kidney γ-GT, restricted to a limited number of methods, which already display little intermethod variability (14). ALAT from porcine heart was not commutable with ALAT in human serum for the methods described (14). These results are in contrast with the results obtained by Gruber et al. (9) who thoroughly compared enzymes of animal origin with enzymes obtained from human tissue (9,11,22). They did not find significant differences in Km values between the human and animal enzymes. The enzymes used to spike the human matrix of Cfas were isolated according to the methods described by Gruber et al.(9). AP and AMYL were not included in these studies. An explanation for this discrepancy may be the fact that Gruber et al. determined the Km values in the purified enzyme preparations whereas the Japanese group determined the Km values in the end product (matrix spiked with enzyme preparations). Furthermore, not all methods used by Gruber et al.(9,11) in the seventies are identical to the methods recommended nowadays.
The use of (recombinant) human enzymes derived from tissue culture has been well documented (13,14,23). Using Seraclear-HE*,* a reduction of the interassay variation of patient γ-GT results from 20% without correction to approximately 4% was achieved. Using pooled human sera interassay variation was reduced to 2.5%. Limited reduction in variation was found using calibrators containing animal enzymes (13). Comparable results were found for ASAT and ALAT (14). In contrast, the AP isolated from a human amnion cell line displayed poor commutability with serum AP and is probably more related to intestinal AP (24). AP in the Enzyme Calibrator has been replaced by a kidney isotype enzyme and in our hands commutability differed minimally from human pooled serum (15). The Enzyme Verifier showed even better commutability than Enzyme Calibrator and much better commutability for Amylase and AP than the Roche and Randox calibrators (15). (B.E.P.B. Ballieux, technical note in preparation).
The interlaboratory (intermethodology) variation in the studies of the WAKO calibrators was assessed by distributing quality control samples also containing human enzymes. This underlines the need not only for a calibrator with human enzymes but also for quality control sera with human enzymes. EQA would be rather spurious when noncommutable sera are used, while laboratories use fully commutable calibration sera.
Not only interspecies variability but also the isotype of the purified enzyme used to spike the matrix may influence the commutability and should always be mentioned. Importantly, the use of placental AP, disregarding its higher stability, is questionable, since the kinetic characteristics differ from those of the liver-kidney-bone isoenzymes, the most common isotypes of AP found in human serum. Furthermore, it has been described that the bovine liver-kidney-bone isotype AP is commutable with human serum AP, while the human placental isotype is not (10,25). However, lack of stability limits the number of alternatives for placental AP and stabilisation of the enzyme also influences commutability (personal communication S. Huber, Bio-Rad, Irvine, California, USA).

Traceability of target values
Instrument dependency is the reason for the differences between the assigned target values in the Calibrator (human) for the Roche Cobas Mira and the Cobas Integra analysers, although identical methods are used. This stresses the fact that values obtained by manual recommended methods and by the derived automated methods are not necessarily identical, since even identical methods used on different analysers do not produce identical results. Therefore, it is essential that traceability of the target values is documented in detail and this information should be readily available from the manufacturer upon request. Interesting in this respect is the fact that the value for alkaline phosphatase is mostly referred to as being measured according to the IFCC method. However, up to now only a provisional IFCC recommendation is available for alkaline phosphatase (26). Furthermore, merely referring to the "DGKC method" is insufficient since new recommendations have been published by the DGKC replacing previous ones. Therefore, the exact reference to the recommended methods should always be given.
All calibrators give separate target values for each method. This implies that patient sera measured by different methods calibrated with their corresponding target value as stated in the insert, do not necessarily give identical results. To overcome intermethod differences of the results of patient sera it is necessary to assign one single target value for each enzyme to the calibrator. In our opinion this target value should be assessed, using the methods recommended most recently by the DGKC (IFCC methods optimized for 37°C) (27-29). The Enzyme committee of the IFCC is also working on recommendations for measurements at 37°C.
The approach of Randox is to establish target values for their calibrator for each method/instrument combination. This will serve to minimize variation between the individual members of each method/

instrument combination, but in our view this will consolidate any differences that exist between the various method/instrument combinations instead of diminishing it. If instrument dependent values are given for a method group these should serve to overcome incomplete commutability of the calibrator between the individual methods.

Conclusions

It can be stated that the use of well defined enzyme calibrators with target values directly traceable to recommended methods will contribute to a decrease of the overall interlaboratory variance for enzyme activity assays and to unification of reference values. If commutability is not fully achievable, method/analyser dependent target values should be assessed by split-sample comparison of the routine method with the recommended manual method using native sera, followed by measuring the calibrator enzyme activity on each of these method/analyser combinations.

Alternatively, the approach suggested in the position paper on national harmonisation may prove useful in the absence of a suitable enzyme calibrator. If target values were assigned to the distributed poolsera at the IFCC enzyme reference laboratory in The Hague, comparability and accuracy would be guaranteed.

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