Interlaboratory Study of the IFCC Method for Alanine Aminotransferase Performed with Use of a Partly Purified Reference Material

Françoise Schiele,1 Jocelyne Muller,1 Elisabeth Colinet,2 Gérard Siest,1 P. Arzogloü,3 H. Brettschneider,4 D. H. Calam,5 Ferruccio Ceriotti,6 Georges Férard,7 Jörg Frei,8 A. Hadjidavasiliou,9 J. C. M. Haackenscheid,10,11 Mogens Hørder,11 F. Javier Gella,12 Robert Rej,13 Ellen Schmidt,14 Anne Vassault,15 and V. Viette16

We present the results of a study on performance of a reference material for alanine aminotransferase (ALT, EC 2.6.1.2) and the corresponding IFCC-approved method in an interlaboratory trial involving 13 laboratories. The ALT material was partly purified from pig heart (specific activity, 150 kU/g) and was essentially free of six potentially contaminating enzyme activities, including aspartate aminotransferase (EC 2.6.1.1). The partly purified ALT was lyophilized in a triethanolamine-buffered matrix, pH 6.4, containing bovine serum albumin and saccharose. Under these conditions, the predicted yearly loss of activity was 0.02% at 4°C and <0.01% at –20°C. The final blank-corrected results of the accepted set of data gave a mean (SD) of 128.5 (5.1) U/L. The among-laboratory SD was 4.6 U/L and the within-laboratory SD was 2.0 U/L. The certified ALT catalytic concentration in the reconstituted material was 129 U/L with a 0.95 confidence interval of ± 4 U/L.

1 Laboratoire du Centre de Médecine Préventive et Centre du Médicament, UA CNRS No 597, 30 rue Lionnois, 54000 Nancy, France.
2 Commission des Communautés Européennes, BCR, rue de la Loi 200, 1049 Brussels, Belgium.
3 University of Salonika, Salonika, Greece.
4 Boehringer Mannheim GmbH, Penzberg, Germany.
5 National Institute for Biological Standards and Control, Potters Bar, UK.
6 Istituto Scientifico, HS Raffaele, Milan, Italy.
7 Université Louis Pasteur, Strasbourg, France.
8 CHU Vaudois, Lausanne, Switzerland.
9 Metasas Memorial Cancer Hospital, Piraeus, Greece.
10 Sint Radboudziekenhuis, Nijmegen, The Netherlands.
11 Odense University Hospital, Odense, Denmark.
12 Universitat Autonoma, Barcelona, Spain.
13 Wadsworth Center for Laboratories and Research, Albany, NY.
14 Medizinische Hochschule, Hannover, Germany.
15 Hôpital Necker—Enfants Malades, Paris, France.
16 Hôpital de la Chaux de-Fonds, La-Chaux-de-Fonds, Switzerland.
17 deceased.
18 Nonstandard abbreviations: ALT, alanine aminotransferase (L-alanine-2-oxoglutarate aminotransferase, EC 2.6.1.2); AST, aspartate aminotransferase (L-aspartate-2-oxoglutarate aminotransferase, EC 2.6.1.1); IFCC, International Federation of Clinical Chemistry; ERM, enzyme reference material; NIBSC, National Institute for Biological Standards and Control; EC BCR, Community Bureau of Reference of the European Communities; DEAE, diethylaminoethyl; BSA, bovine serum albumin; CM, carboxymethyl; PBE, polybuffer exchanger; PP, pyridoxal 5'-phosphate; and SFBC, Société Française de Biologie Clinique.

Received December 10, 1990; accepted July 24, 1992.

Serum alanine aminotransferase (ALT, EC 2.6.1.2) is, along with aspartate aminotransferase (AST, EC 2.6.1.1), one of the most frequently measured enzyme activities in clinical laboratories.17 The importance of ALT has also increased because it is commonly determined in blood banks to identify blood samples potentially contaminated with non A--non B hepatitis virus (hepatitis C).

In 1980, the Expert Panel on Enzymes of the International Federation of Clinical Chemistry (IFCC) proposed a method for measuring ALT activity in human serum; this method was officially approved in 1985 (1). International cooperation has led to enzyme reference materials (ERMs) being available for most of the currently used enzymes in clinical chemistry. The procedure for value assignment for catalytic concentration of the ERMs uses the most accurate and reliable measurement technique available through strictly defined and controlled interlaboratory studies. However, there is relatively little information concerning the transferability of the IFCC ALT method and, to our knowledge, no interlaboratory study using purified ALT has been performed, although this was done for AST (2, 3) and alkaline phosphatase (4, 5).

Therefore, we report here the results of a study of an ERM for ALT where the IFCC-recommended method was used in an interlaboratory trial run under the auspices of the Commission of the European Communities. The objectives of this study were to test the possibility of a partly purified ALT being an ERM and to assign a value to the material by using the IFCC method for ALT measurement through an interlaboratory study.

Materials and Methods

Instrumentation

The spectrophotometers used in the different participating laboratories, grouped by temperature-monitoring system, were (a) those with a thermistor in the reaction solution: Beckman Model DU 8 (bandpass, 0.5 nm; Beckman Instruments, Fullerton, CA); Cary Model 219 (bandpass, 1 nm; Varian Instrument Div., Palo Alto, CA); Eppendorf 1101 M (bandpass <1 nm; Eppendorf Geratetubag Netherlert Hinz GmbH, Hamburg, Germany); Kontron Uvikon Models 860, 930, and 940 (bandpass, 2 nm; Kontron Instruments, Everett, MA);
and Shimadzu UV 190 (bandpass, 2 nm; Shimadzu Europa GmbH, Duisberg-Grossenbaum, Germany); (b) those with a thermistor mounted in the wall of the cuvette holder: Cary Model 219 (bandpass, 1 nm) and Gilford Stasar III (bandpass, 8 nm; Gilford Instrument Labs. Inc., Oberlin, OH); and (c) one with a thermistor in water bath: Beckman Model 25 (bandpass 1 nm).

Chemicals

Potassium phosphate, disodium EDTA, ammonium sulfate, potassium chloride, imidazole, glycine, histidine, Tris, saccharose, and triethanolamine were obtained from Merck (Darmstadt, Germany). Dithiothreitol and bovine serum albumin (BSA; purity 98–99%, cat. no. A-7030) were purchased from Sigma Chemical Co. (St. Louis, MO). Diethylaminoethyl (DEAE)-tris-acyl and carboxymethyl (CM)-tris-acyl were from Industrie Biologique Française (Paris, France). Ultrogel ACA 34 was purchased from LKB Produkter AB (Bromma, Sweden). Polybuffer exchanger (PBE) and cyanogen bro- mide-activated Sepharose 4-B were obtained from Pharmacia (Uppsala, Sweden). Sodium chloride was from Prolabo (Paris, France), and pyridoxal 5’-phosphate (PP) and 2-oxoglutaric acid were from Boehringer Mannheim (Meylan, France).

Chemicals used for measuring catalytic concentration of ALT (provided by Boehringer Mannheim GmbH, Mannheim, Germany) were supplied to all participating laboratories. One batch of each reagent (Tris buffer, L-alanine, 2-oxoglutaric acid, disodium NADH Grade I (purity, 100%), lactate dehydrogenase from pig skeletal muscle in a glycerol matrix, and PP) was used for the study. In addition, each laboratory was provided with a single batch of d-alanine (purity >99%, d:L >99.5:0.5; Fluka AG, Buchs, Switzerland) for sample blank reac- tion measurements. The purity of the reagents was controlled and was shown to meet the requirements of the IFCC-approved method (1). Reagent mixtures were prepared in each laboratory according to the IFCC method.

To assess the linearity of the spectrophotometers in the absorbance range 0.39–1.64 at 339 nm and to reveal any gross errors in their accuracy, each laboratory used a commercially stabilized solution of NADH (UV-Trol; BioMérieux, Charbonnières-les-Bains, France).

Methods

We measured ALT activity during the preparation and characterization of the ALT material with a Cary Model 219 spectrophotometer or with a Cobas Bio centrifugal analyzer (Hoffmann-La Roche, Basel, Switzerland) with kits from Boehringer Mannheim, at 30 °C (7). Protein concentrations were determined by the method of Lowry et al. (6) with BSA as standard.

We examined the partly purified ALT for various contaminating enzyme activities by using commercially available kits. We used kits from BioMérieux for measurements of alkaline phosphatase (EC 3.1.3.1), creatine kinase (EC 2.7.3.2), lactate dehydrogenase (EC 1.1.1.27), and AST activities according to the methods recommended by the Société Française de Biologie Clinique (SFBC) (7) and for acid phosphatase (EC 3.2.3.2) activity according to Hillmann’s method (8). γ-Glutamyltransferase (EC 2.3.2.2) activity was determined with a kit from Boehringer Mannheim (9).

Preparation of the ALT Material

ALT was partly purified from pig heart according to the procedures of Kojima (10) and Naguchi et al. (11), as modified in our laboratory. One part tissue (200 g) was homogenized with three parts phosphate buffer (50 mmol/L, pH 6.0) containing, per liter, 0.2 mmol of EDTA, 0.1 mmol of dithiothreitol, 0.05 mmol of PP, and 5 mmol of 2-oxoglutaric acid in a Waring Blender at maximal velocity for 3 min (3 × 1 min) at 4 °C. The mixture was heated to 65 °C and kept at that temperature for 1 min, immediately cooled at 4 °C in an ice bath, and centrifuged at 12 000 × g for 20 min at 4 °C. The supernate was precipitated with 20–55% ammonium sulfate saturation followed by centrifugation at 12 000 × g for 20 min at 4 °C. The pellet was solubilized in a solution containing imidazole (25 mmol/L, pH 7.6) and EDTA (5 mmol/L) and dialyzed overnight at 4 °C against the same buffer.

The material was chromatographed at room temperature on a 5 × 40 cm column of DEAE-tris-acyl, eluted with a linear gradient of KCl (0–0.3 mol/L) in the imidazole buffer, and dialyzed at 4 °C against phosphate buffer (50 mmol/L, pH 6.8).

The active fractions were concentrated at room temperature by ultrafiltration in a stirred cell (Amicon Corp., Lexington, MA) with a PM 10 membrane, dialyzed at 4 °C against a histidine buffer (25 mmol/L, pH 6.2), and filtered at 4 °C on a 2.5 × 100 cm column of Ultrogel ACA 34 equilibrated with Tris HCl buffer (25 mmol/L, pH 7.5). Next the material was chromatographed at room temperature on a 1 × 6 cm immunoaffinity column prepared from anti-cytoplasmic AST antibodies that we produced in our laboratory and bound to a bromide cyanogen-activated Sepharose-4B column in NaHCO₃ buffer (100 mmol/L, pH 8.3) containing NaCl, 50 mmol/L. After equilibrating the column with phosphate buffer (100 mmol/L, pH 7.0) containing NaCl at 150 mmol/L, we collected directly the fractions containing ALT activity by elution with the same buffer. The remaining AST in the preparation was retained on the column and eluted with a glycine HCl buffer (20 mmol/L, pH 2.5).

We analyzed for potentially contaminating enzymes at different stages during the purification process. The partly purified ALT was added to the following matrix to yield a catalytic concentration of ~130 U/L per liter, 30 g of BSA, 10 g of saccharose, and 50 mmol of triethanolamine hydrochloride, pH 6.4. Before dilution, we measured the activities of possible contaminating enzymes in the BSA. The liquid material was frozen and shipped on solid carbon dioxide to the National Institute for Biological Standards and Control (NIBSC, Potters Bar, UK) for lyophilization. The enzyme solution was thawed at 4 °C, then filtered at the same temperature.
through two 0.22-μm pore-size membrane filters (Sartorius Ltd., Epsom, UK). It was dispensed at 4 °C into ampules at a nominal volume of 1.0 mL per ampule. The ampules were processed as a single batch essentially as described by Campbell (12) by using a Minifast freezedrier (Edwards High Vacuum, Crawley, UK). Lyophilization commenced at a shelf temperature of −50 °C. When it was complete, the bath was submitted to secondary desiccation over phosphorus pentoxide for 5 days. The ampules were then filled with pure, dry nitrogen and sealed by fusion of the glass. The total number of ampules (1 mL) so prepared was 1768.

Characterization of the ALT Material

The precision and accuracy of dispensing the ALT liquid material was monitored by weighing 34 ampules taken at regular intervals throughout the filling procedure and before and immediately after dispensing the 1-mL volumes. The residual moisture content of the lyophilized material was determined in three ampules by an automated Fischer method (13). The uniformity (ampule-to-ampule variability) of the lyophilized product was assessed by measuring ALT catalytic concentration in 20 different ampules picked at random.

The long-term stability studies of the lyophilized preparation were coordinated by NIBSC and were conducted in the same manner as for γ-glutamyltransferase Certified Reference Material 319 by using an accelerated thermal degradation procedure (14). ALT catalytic concentration was measured after 65 and 189 days of storage at different temperatures. Each ampule was assayed in duplicate with and without PP, and the predicted loss of activity was calculated by NIBSC (14). The stability of the reconstituted material at room temperature during a working day was examined by measuring ALT catalytic concentration in duplicate by the SFBC method (7) with a Cobas Bio centrifugal analyzer at 1-h intervals for 8 h.

We examined some of the major kinetic properties of the partly purified ALT. We determined the apparent Michaelis constants (Km) for L-alanine and 2-oxoglutarate at 30 °C by using the IFCC method except for varying the substrate concentration.18 Simultaneous measurements were performed in duplicate with the ALT material and with a pooled specimen of human serum. L-Alanine concentrations were 25–700 mmol/L, and 2-oxoglutarate concentrations were 1–24 mmol/L. We also tested the effect on ALT activity of preincubation with PP, 0.1 mmol/L, for 15 min.

Interlaboratory Study

Thirteen laboratories were involved in the interlaboratory study: three in France, two in Germany, two in Greece, and one each in Denmark, Italy, Spain, Switzerland, The Netherlands, and the United States. All laboratories were familiar with the IFCC method for measuring catalytic concentration of ALT because they had been involved in a preliminary analytical run during a feasibility study with a pilot batch of ALT material.

Each participating laboratory was provided with five ampules of ALT material, a set of reagents, and a set of documents, including a copy of the IFCC method for measuring ALT catalytic concentration, a protocol for reconstituting the lyophilized material, a protocol for checking instrumentation, a form requesting details concerning instruments and ALT-material reconstitution, and a sheet for reporting results. Each laboratory was required to perform duplicate analyses on 3 different days, a different ampule being freshly reconstituted and analyzed on each day.

The Community Bureau of Reference of the European Communities (EC BCR) collected all data for statistical evaluation.

Results

ALT Material

Our procedure for purification of ALT from pig heart yielded a 470-fold-purified enzyme with a specific activity of ~150 kU/g protein (Table 1). γ-Glutamyltransferase and lactate dehydrogenase activities were not detectable after the DEAE-tris-acryl chromatography. After the gel filtration on Ultrogel ACA 34, activities of alkaline and acid phosphatases were not measurable; creatine kinase activity was 1 U per 100 U of ALT. The final product after the immunoaffinity chromatography with anti-cytoplasmic AST antibodies contained only 0.1 U of AST activity per 100 U of ALT.

The apparent Km values obtained for L-alanine were 49.1 and 27.9 mmol/L for the pig-heart ALT and the human serum pool, respectively. For 2-oxoglutarate, the apparent Km values were 0.41 and 1.26 mmol/L, respectively.

We observed an increase of ~3.5% of the ALT activity after preincubating the partly purified enzyme with PP.

Table 1. Partial Purification of ALT from Pig Heart

<table>
<thead>
<tr>
<th>Step Description</th>
<th>Total catalytic activity, U</th>
<th>Total protein mass, mg</th>
<th>Specific catalytic activity, kU/g</th>
<th>Purification, n-fold</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>3000</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>2120</td>
<td>6775</td>
<td>0.31</td>
<td>(1)</td>
<td>100</td>
</tr>
<tr>
<td>Ptn with (NH₄)₂SO₄</td>
<td>1700</td>
<td>1180</td>
<td>1.44</td>
<td>4.7</td>
<td>80</td>
</tr>
<tr>
<td>Chromatography on DEAE-tris-acryl</td>
<td>1400</td>
<td>141</td>
<td>9.93</td>
<td>32</td>
<td>66</td>
</tr>
<tr>
<td>Chromatography on CM-tris-acryl</td>
<td>740</td>
<td>46</td>
<td>16.1</td>
<td>52</td>
<td>35</td>
</tr>
<tr>
<td>Gel filtration on Ultrogel ACA 34</td>
<td>555</td>
<td>15</td>
<td>36.8</td>
<td>119</td>
<td>26</td>
</tr>
<tr>
<td>Immunoaffinity chromatography</td>
<td>470</td>
<td>3.2</td>
<td>147</td>
<td>474</td>
<td>22</td>
</tr>
</tbody>
</table>

* Percent of total catalytic activity.

b For 200 g of fresh material.

---

18 In the published IFCC method (p. 483, ref. 1), the catalytic concentration of lactate dehydrogenase (solution V) should be 2.52 x 10⁹ nkat × L⁻¹.
within-ampule. The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase atine 0.1 tion (SD analysis in lytic less

The mass transferase at
Statistical analysis (15) yielded no outlying value among the set of results accepted on technical grounds. Because the analysis of variance showed that the between-laboratory variability was the major source of variability, each laboratory’s mean value (and not the individual results) was used to calculate the certified value and uncertainty (16). The certified ALT catalytic concentration of the reconstituted (with 1 mL of distilled water) material and the corresponding uncertainty at the 0.95 confidence level was 219 ± 4 U/L. This material is now available through the EC BCR as CRM 426.

Discussion

In this interlaboratory study we used partly purified ALT from pig heart. Pig heart ALT was said to have catalytic properties very similar to those of the human serum enzyme (17), as was also shown by our results. Particular attention was paid to the absence of potentially contaminating enzyme activities. Although the specific activity that we obtained (150 kU/g protein) was far from that reported for preparations from the same organ by Saier and Jenkins (18) and Kojima (19) (i.e., 498 and 340 kU/g protein, respectively), our preparation was free of the main potentially contaminating enzyme activities. The major difficulty was, in fact, to obtain the partly purified ALT free of AST, because these two enzymes have very similar chemophysical properties (i.e., isoelectric point and relative molecular mass). This separation was achieved by using immunochromatography with antibodies directed against the cytoplasmic AST isoenzyme.

Another difficulty was to obtain a stable material. Some interlaboratory studies described difficulties linked to the instability of the enzyme preparations (19, 20). We chose a triethanolamine-buffered matrix at pH 6.4, containing BSA and saccharose. This pH value was selected because purified rat-liver ALT includes sulfhydryl groups, some of them directly involved in the enzyme activity (21). Similarly, a relatively acidic pH value was shown to ensure good storage stability of creatine kinase isoenzymes (22), which also contain thiol groups. The partly purified ALT was predominantly saturated with its cofactor, as shown by the fact that incubation with PP increased its activity by <5%; this increase was completed within the 10-min preincubation time required by the IFCC method (1). In addition, previously described interaction between albumin present in the AST RM 8430 samples and PP was said to be responsible for a 339-nm absorbance change both in the overall reaction and in the sample blank reaction for 15 min (3, 23). Therefore, we preferred not to add PP in the matrix to avoid possible sources of imprecision in measuring ALT catalytic concentration, specifically for blank reactions.

The ALT material appeared suitable in terms of stability, uniformity, and contaminating enzyme activities for use in an interlaboratory trial. The ampule-to-ampule variability for ALT catalytic concentration in the reconstituted material was 1.3%. The predicted

### Table 3. Final Corrected Results for ALT Catalytic Concentration from Participating Laboratories

<table>
<thead>
<tr>
<th>Lab</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Mean (SD), U/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>118.9</td>
<td>120.4</td>
<td>116.8</td>
<td>120.0 (2.28)</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>123.4</td>
<td>118.9</td>
<td>121.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>123.7</td>
<td>122.1</td>
<td>122.3</td>
<td>122.7 (0.68)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>123.3</td>
<td>122.9</td>
<td>122.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>124.1</td>
<td>122.6</td>
<td>124.1</td>
<td>124.0 (0.80)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>124.1</td>
<td>124.1</td>
<td>125.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>122.5</td>
<td>126.8</td>
<td>126.5</td>
<td>125.5 (3.49)</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>122.7</td>
<td>130.1</td>
<td>122.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>128.0</td>
<td>130.3</td>
<td>131.5</td>
<td>129.7 (2.05)</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>128.4</td>
<td>127.6</td>
<td>132.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>130.8</td>
<td>129.0</td>
<td>129.5</td>
<td>129.8 (2.49)</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>131.2</td>
<td>125.4</td>
<td>132.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>129.6</td>
<td>127.7</td>
<td>131.5</td>
<td>129.9 (2.82)</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>131.5</td>
<td>125.8</td>
<td>133.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>133.1</td>
<td>130.8</td>
<td>132.9</td>
<td>132.5 (1.06)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>133.3</td>
<td>131.6</td>
<td>133.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>135.8</td>
<td>131.8</td>
<td>130.6</td>
<td>132.6 (2.14)</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>134.6</td>
<td>130.6</td>
<td>132.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>135.5</td>
<td>136.3</td>
<td>129.6</td>
<td>132.8 (4.13)</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>137.1</td>
<td>131.0</td>
<td>127.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>134.2</td>
<td>136.2</td>
<td>135.1</td>
<td>134.4 (1.20)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>132.6</td>
<td>134.1</td>
<td>134.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>128.5 (5.06)</td>
<td>3.9</td>
</tr>
</tbody>
</table>

### Table 4. Comparison of the Sources of Variability in the Preliminary and Final Interlaboratory Studies

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Preliminary study*</th>
<th>Final study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD of activity, U/L</td>
<td>CV, %</td>
</tr>
<tr>
<td>Between-lab</td>
<td>8.10</td>
<td>7.0</td>
</tr>
<tr>
<td>Between-day</td>
<td>3.31</td>
<td>2.8</td>
</tr>
<tr>
<td>Within-day</td>
<td>1.59</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Variance components were calculated from duplicate measurements of one ampule on 3 days in 13 participating laboratories for the preliminary study and 11 laboratories for the final study. Degrees of freedom for mean squares for the preliminary study: between lab, 12; between day, 26; and overall, 77. Degrees of freedom for mean squares for the final study: between lab, 10; between day, 22; and overall, 65.

*The preliminary interlaboratory study used a partly purified material prepared in the same way as the material used for the final study and having a similar catalytic concentration (116.5 U/L).
change in ALT catalytic concentration in the lyophilized material at \(-20^\circ C\) was \(<0.01\%\) per year.

The ALT catalytic concentration of the material was determined by using the IFCC-approved method (1). All the participating laboratories were involved in a preliminary trial. This preliminary trial pointed out some problems encountered with the implementation of the IFCC-approved method, in particular with the purity of reagents, especially the D-alanine, and with participating laboratories not being sufficiently familiar with the method. Possible spurious values of sample blank caused by contamination of D-alanine with L-alanine were previously noted by Okorodudu et al. (24). We measured the overall sample blank reaction with D-alanine that was \(\approx 99\%\) pure from six different manufacturers. The values obtained ranged from 2.1 to 11.4 U/L. We decided to use one lot of reagents for this interlaboratory trial and to test all reagents' purity. In addition, to ensure that the laboratories were themselves sufficiently familiar with the IFCC method, the participants in this second trial were the same as those involved in the feasibility study. However, most of the published interlaboratory data from IFCC methods has needed several trials before acceptable between-laboratories variations were obtained.

The importance of preinstrumental and instrumental errors in the measurement of catalytic concentration of enzymes has been stressed in many other interlaboratory studies (2-5, 15, 25). We used a well-defined protocol to minimize and possibly to identify all the sources of variation in the measurement procedure. The volumes delivered by the sampling pipettes, derived from the weight of distilled water delivered during pipetting (mL = mass/density corrected for temperature), ranged from 198.5 to 202.0 \(\mu\)L (99.25-101.00% of the expected value). Each laboratory used its own sampling volume when calculating the ALT catalytic concentration. The variation in the reconstitution of the lyophilized samples was estimated to be \(\pm 0.86\%\). The total variability of the results accepted for certification was 3.9%. Bowers et al. (26) obtained a CV of 7.4% but for a much lower mean value (24.2 U/L) and without sample blank correction with lyophilized human serum material NBS SRM 909 (National Institute of Standards and Technology, Gaithersburg, MD). They identified between- and within-laboratory CVs, including the day-to-day variability, of 6.5% and 3.5%, respectively. In this study the respective CVs were 3.6% and 1.8% and are comparable with those obtained in other interlaboratory studies involving IFCC methods. The between-laboratory CVs were 2.2% for AST (3), 2.6-6.3% (4) and 4.3% (5) for alkaline phosphatase, and 3.1% for \(\gamma\)-glutamyltransf erase (15). The within-laboratory CVs, including the day-to-day variability, were 1.2% for AST, 1.2-3.6% and 1.6% for alkaline phosphatase, and 1.6% for \(\gamma\)-glutamyltransferase.

The small within-laboratory CV (1.8%) including the day-to-day variation (CV = 1.5%) is evidence of the excellent reproducibility achievable by the IFCC method. The variation between laboratories (CV = 3.6%) reflects the current state of the art. The consistency of the results between laboratories in several different countries, including the United States, clearly demonstrates the transferability of the IFCC-approved method for measuring ALT catalytic concentration.

We are very indebted to R. Gaines Das (National Institute for Biological Standards and Control, Potters Bar, UK) for calculating the predicted degradation rates of the material. We thank C. Cossey and M. P. Recouvreur for their excellent technical assistance.

References


2370 CLINICAL CHEMISTRY, Vol. 38, No. 12, 1992