A Unifying Reference System for Clinical Enzymology: Aspartate Aminotransferase and the International Clinical Enzyme Scale

George N. Bowers, Jr.,¹ and Robert B. McComb

A review of methodology for determining aspartate aminotransferase (ASAT; EC 2.6.1.1), including recent national and international recommendations, indicates that standardization of methodology alone will not bring interlaboratory compatibility of ASAT results. We propose that an additional component to standardization is needed, namely, enzyme reference materials. Furthermore, we suggest that stable, well-defined ASAT materials from human sources are currently available. These primary reference materials and the state-of-the-art IFCC Reference Method for ASAT provide the basis for a unifying reference system for ASAT. Given such a reference system, we propose a practical way to promote compatibility of currently incompatible numerical results for ASAT through the use of one ASAT scale of units, the "International Clinical Enzyme Scale." This scale-unification concept would permit all current methods, instruments, and temperature choices to be used for ASAT determinations in the daily working laboratory. We present illustrative examples and demonstrate the unique ability of this concept to promote compatibility of the ASAT results from numerous laboratories using many different ASAT methods.

Additional Keyphrases: enzyme reference materials · enzyme reference methods · enzyme activity · enzyme standardization · interlaboratory performance in clinical enzymology

Large interlaboratory proficiency surveys for enzymes reveal extremely wide ranges in the numerical values reported from laboratory to laboratory for the same specimen. The numerical values are frequently so dissimilar that it is often impossible to recognize them as having any relation to one another, and they can only be described as grossly incompatible. For example, aspartate aminotransferase (ASAT) results from 4000 laboratories for sample C-7 in the 1983 College of American Pathologists (CAP) Comprehensive Chemistry Survey (1) were assigned to one of 36 ASAT method groupings depending upon reagent, instrument, and temperature combinations.² The mean values for these method groupings ranged from 61.5 to 152.2 U/L. Given the stated standard deviations of the two extreme means, the reported values, exclusive of outliers, must have extended from about 48 to about 166 U/L.

This unsatisfactory situation is correctable. Using ASAT as the enzyme model in this paper, we demonstrate a relatively simple and direct way to convert incompatible ASAT numerical values to compatible values by use of a single scale, the International Clinical Enzyme Scale (ICES) (2). We also show that ICES as applied to ASAT is not a sudden new approach but is carefully built upon a well-defined existing reference technology for ASAT.

The Maturation of ASAT Methodology

There have been intense efforts over the years (3–10) to "optimize" the measurement of ASAT activity by steadily improving on the one basic ultraviolet-absorbance method described by Karmen (11). In the primary enzyme reaction ASAT catalyzes the transfer of an amino group from L-aspartate to 2-oxoglutarate to yield L-glutamate and oxalacetate. In the secondary (indicator) reaction, catalyzed by malate dehydrogenase (MDH), the oxalacetate is reduced to L-malate as NADH is oxidized to NAD⁺. In this continuous kinetic method, the timed absorbance change at 339 nm (da/dt), related to the decreasing concentration of NADH, is the physical property that is proportional to the catalytic activity concentration of the ASAT in the sample.

Originally, Karmen reported activity directly in terms of the change in milliabsorbance units per minute at 340 nm in a defined sample and reagent mixture. No chemical or enzyme reference material was used as the calibration standard, and the activity value thus depended totally on the photometric accuracy of the instrument used. This unsatisfactory reliance on an instrument's unknown photometric performance went unquestioned for many years (12). Because NADH is the key chemical reference material (or

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² Nonstandard abbreviations: ASAT, aspartate aminotransferase (EC 2.6.1.1); L-aspartate:2-oxoglutarate aminotransferase; EPE, Expert Panel on Enzymes; ICES, International Clinical Enzyme Scale; IFCC, International Federation of Clinical Chemistry; MDH, malate dehydrogenase; NBS, National Bureau of Standards; NCCLS, National Committee for Clinical Laboratory Standards; PP, pyridoxal 5'-phosphate; SRM, Standard Reference Materials; CAP, College of American Pathologists; NCCLS, National Committee for Clinical Laboratory Standards; and CDC, Centers for Disease Control.
calibration standard) but is not readily available as a stable high-purity material, molar absorptivity values reported in the literature must be used (13, 14). Based on these literature values and the appropriate correction factors for the conditions found in the final ASAT reaction mixture, we estimate this value and its overall uncertainty to be 6.32 × 10^3 ± 0.03 × 10^3 L mol⁻¹ cm⁻¹. In addition, one must rigorously standardize the entire instrumental measurement system (15) in order to guarantee unerring traceability to the SI Base Units.

The introduction of colorimetric 2,4-dinitrophenylhydrazine (16, 17) and azoene dye (18) methods for ASAT, in which chemical reference materials were used, only added to the confusion because the new units used to express results for ASAT activity were numerically incompatible with the reference intervals for the Karmen method, which had become widely accepted in clinical practice. Two factors helped reverse the ever-increasing proliferation of numerically incompatible enzyme results that had developed by 1960, not only for ASAT but also for the other clinically determined enzymes. First, the international unit (U), micromole per minute, was introduced by joint efforts of IUPAC and the IUB (19) as a systematic means of reporting enzyme activity. Most methods developed since the early 1960s for measuring the catalytic activity concentration of enzymes in human serum are now expressed in terms of U/L although a newer, more SI-consistent katal unit (kat), mole per second, has now been introduced (20). Second, methods based on the coupled MDH–NADH reaction of Karmen have progressively replaced the colorimetric methods. Ultraviolet methods based on MDH–NADH are currently used in about 95% of all U.S. clinical laboratories (21).

This trend toward uniformity of ASAT methodology progressed slowly but steadily during the 1970s as national and international groups developed and published recommendations for ASAT reference methods. Although there had been significant changes towards the use of more "optimized" ASAT methodology as early as 1960 (5), it was not until 1972 that ASAT reference methods were officially endorsed by national organizations of laboratory professionals (22, 23). Since 1972, 10 national recommendations for ASAT reference methods have been published (22–31). The first truly international method for any enzyme was that for ASAT recommended by the Expert Panel on Enzymes (EPE) of the IFCC in 1976 (32). This EPE/IFCC reference method for ASAT embodied the high state-of-the-art measurement technology prescribed for IFCC Reference Methods (33). This "general considerations" document has now been officially accepted by a majority vote of the entire international membership of IFCC as a fully endorsed IFCC recommendation.

In each of the 10 national (and the one international) recommendations for ASAT reference methods, the MDH–NADH-coupled spectrophotometric procedure of Karmen was retained as the basic method. As compiled in Table 1 from a recent review on ASAT (21), the final reaction conditions for the national recommended methods for ASAT are quite similar to the ASAT recommendations of the EPE/IFCC, the only differences that cause major changes in the catalytic activity concentration being (a) the presence or absence of pyridoxal 5'-phosphate (PP) and (b) the patent

<table>
<thead>
<tr>
<th>Final reaction conditions</th>
<th>National</th>
<th>IFCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (°C)</td>
<td>1–25.0, 5–30.0, 2–37.0, 2–30</td>
<td>30.0</td>
</tr>
<tr>
<td>or 37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>2–7.4, 1–7.6, 1–7.7, 6–7.8</td>
<td>7.80</td>
</tr>
<tr>
<td>Buffer type and concn (mmol/L)</td>
<td>2–PO₄, 8–Tris (2–20, 1–60,</td>
<td>80 Tris</td>
</tr>
<tr>
<td></td>
<td>4–80, 1–100</td>
<td></td>
</tr>
<tr>
<td>L-Aspartate concn (mmol/L)</td>
<td>1–175, 8–200, 1–240</td>
<td>240</td>
</tr>
<tr>
<td>l-Oxoglutarate concn (mmol/L)</td>
<td>1–10, 8–12, 1–15</td>
<td>12</td>
</tr>
<tr>
<td>PP concn (mmol/L)</td>
<td>4–0, 1–0.03, 4–0.10, 1–0.14</td>
<td>0.10</td>
</tr>
<tr>
<td>NADH concn (mmol/L)</td>
<td>1–0.13, 3–0.15, 1–0.16, 5–0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>MAD acty (U/L)²</td>
<td>1–167, 7–600, 1–400</td>
<td>430</td>
</tr>
<tr>
<td>LDH acty (U/L)³</td>
<td>3–0, 2–200, 1–500, 3–600, 1–1200</td>
<td></td>
</tr>
<tr>
<td>Volume fraction</td>
<td>2–NS, 1–0.067, 3–0.083, 1–0.010, 2–0.12, 1–0.13</td>
<td>0.083</td>
</tr>
</tbody>
</table>

* Karmen (11) MAD/NADH continuous spectrophotometric techniques.
* Activity under conditions defined in the recommendations.

Table 1. Ten National and the EPE/IFCC Recommended Methods for ASAT

![Aspartate aminotransferase scales](image)

**Fig. 1. Aspartate aminotransferase scales**

Each horizontal line represents one of seven distinct scales for ASAT activity measured at four reaction temperatures and the use or absence of pyridoxal 5'-phosphate. Intersection of the horizontal line with the sides of the triangle represents the upper and lower reference limits, expressed in U/L. Although these reference values vary among sets of populations, the values for any one set are linearly related to each other. We propose that all ASAT scales be systematically converted to the International Clinical Enzyme Scale (ICES) so that all ASAT results will be numerically compatible.
spread of the values obtained, owing to the many scales used to report ASAT results. Although each of these scales is the logical outcome of many rational decisions involving key considerations of method or temperature, these independent scales predictably give incompatible numerical values. Scale unification to ICES can convert these grossly incompatible results into highly comparable results. To achieve this, we must create the formal international agreements to support an ASAT reference system that includes both ASAT reference method technology and well-characterized ASAT reference materials.

Primary ASAT Reference Materials

To serve as a primary enzyme reference material for use in clinical enzymology, a material must resemble the counterpart enzyme in human serum and exhibit long-term stability. In addition, selected laboratories throughout the world must be capable of producing "identical" batches of the material. The existence of such a material must then be widely recognized and its properties and method of manufacture formally defined by a universally accepted "enzyme authority." Finally, the "enzyme authority" must certify the catalytic activity concentration values assigned to the lots of primary enzyme reference materials.

Actually, a suitable reference material for ASAT has been available for some time. In the early 1970s, Rej et al. described the manufacture of high-purity stable ASAT from human erythrocytes (36). Subsequently, Burtis et al. have prepared numerous batches of primary ASAT reference material from erythrocytes and have carefully characterized a matrix that promotes long stability of the ASAT (37, 38). Both groups have used these ASAT reference materials to study the ability of clinical service laboratories to measure this enzyme's activity under daily operating conditions (37–39). This material has now been recognized by a U.S. national authority as a suitable reference material (40).

Apart from the use of high-quality enzyme materials for daily quality-control monitoring and interlaboratory proficiency surveys, enzyme reference materials with assigned activity values have increasingly been suggested as calibration standards. Review of the literature on enzyme standardization for the past two decades demonstrates that many investigators (45–55) have contributed to the slowly but progressively evolving body of knowledge that now supports the systematic use of ASAT reference materials in conjunction with ASAT reference methods to create an ASAT reference system (2, 61, 66). Let us now examine this concept of reference systems to see how they promote and maintain accuracy and (or) compatibility of results.

Reference Systems in the Clinical Laboratory

As a result of a national conference held in 1977 (67), the U.S. clinical laboratory community has created a National Reference System for the Clinical Laboratory (NRSCL)6 to promote interlaboratory accuracy of results based on the "true value" or, when the "true value" cannot be defined for analytes such as enzymes, on the interlaboratory compatibility of results. The NRSCL rests upon a hierarchy of interrelated Definitive Methods (69), Reference Methods (68), Certified Reference Materials (70), Calibrators, and Quality-Control Materials (71, 72) to achieve these accuracy and compatibility goals. Figure 2 illustrates the "idealized" interrelationships among the technical components (units, materials, and methods) of this accuracy-based reference system (73, 74).

In October 1978, the NRSCL Council gave ASAT the highest priority of any enzyme and ranked it 10th of the top 25 analytes on the Priority Analyte Listing (75). Fortunately, the IFCC Reference Method for ASAT (32) has been developed, validated, and transferred to other laboratories (38, 60) in a manner entirely consistent with the NRSCL Guidelines for Reference Methods (69). In 1983, the NRSCL Council "accepted" the IFCC Reference Method for ASAT into the NRSCL and the Board of Directors of NCCLS has approved its circulation to the NCCLS membership for review and comment as a Proposed Method for ASAT. In addition, the NRSCL Council has also formally "accepted" the specifications for the preparation of human erythrocyte ASAT (40) as a reference material that meets the NRSCL guidelines for Certified Reference Materials (70) and is now also undergoing NCCLS review.

In parallel with prior actions on several dozen nonenzyme analytes, these NRSCL and NCCLS actions affirm the importance of an ASAT reference method and a stable, well-defined primary ASAT reference material. Together, these actions represent a clear endorsement of the concept of an ASAT reference system by two senior policy and review bodies responsible for the voluntary consensus standards process within the American clinical laboratory community.

A Unification Proposal

We in clinical laboratory medicine have created too many scales for reporting ASAT values and have been slow to

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4 When serum-based enzyme calibrators were first introduced, there was a tendency to call them "enzyme standards in serum" (41). This nomenclature is incorrect, as pointed out in the classic article on standardization by Radin (42) and by the IFCC Expert Panel on Quality Control and Calibration (43). The use of the phrase "enzyme standards in serum" was officially discouraged by the American Association for Clinical Chemistry through a statement originating from its Standards Committee and endorsed by its governing body in 1967 (44). Even with the subsequent advent of highly purified and well-characterized human ASAT materials (36, 37), these unacceptable ASAT reference methods by which to measure their activity (22–32), these products are preferably designated as primary or secondary enzyme reference materials (or calibration standards).

6 Known formerly (before March 1984) as The National Reference System for Clinical Chemistry.

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Fig. 2. Relationship among the different technical components of an "idealized" reference measurement system (from ref. 73 by permission)
work at scale unification. Too many scales and a long delay in unification is not a problem unique to clinical enzymology: for centuries, the field of thermometry had similar problems with many incompatible measurements due to multiple instruments and many unrelated scales (76). For example, some thermometers made in Europe between 1750 and 1850 contained as many as 18 different scales! Through international agreements on units (Celsius and Kelvin), methods (standard interpolation instruments), and materials (13 defining fixed points), the International Practical Temperature Scale (IPTS) was introduced, and through periodic technical revisions it has evolved slowly and systematically to its present highly functional state (77).

Like the IPTS for temperature, the measurement of the catalytic activity concentration of an enzyme in serum must also rest squarely upon the natural properties of stable, well-characterized enzyme reference materials and state-of-the-art reference methods. To this end, we have proposed (2) an enzyme reference system that unifies currently incompatible numerical enzyme values by the use of one scale, the ICES. The primary human ASAT reference materials mentioned earlier would carry ASAT/ICES values that permit the calibration of all other methods to ASAT/ICES. The ASAT/ICES-certified values would be assigned to the primary ASAT reference materials within a network of special ICES reference laboratories by use of the IFCC Reference Method for ASAT (32) at the primary gallium reference temperature of 29.772 °C (77–81). This initial value assignment is the crucial step in the further transfer of certified ASAT primary reference materials into industrial calibration laboratories.

The Role of Secondary ASAT Reference Materials

We anticipate that much of the enzyme standardization performed in the bulk of the world's clinical laboratories will make use of secondary enzyme reference materials. These secondary reference materials will be assigned ASAT/ICES values, but in most cases the activities would be derived by direct comparison with concurrently analyzed calibrated primary reference materials. Through such comparison calibrations with certified primary ASAT reference materials, stable secondary ASAT reference materials and related ASAT calibrators (71) and ASAT quality-control materials (72) would receive their ASAT/ICES value assignments in industrial calibration laboratories just as they do today. These secondary ASAT materials would then flow into the numerous clinical service laboratories around the world for calibrating the daily working instruments, again just as they do today. Without changing a single method or reaction temperature anywhere in the daily working laboratories (except, of course, to calibrate in terms of ASAT/ICES), all results would be reported directly in terms of ASAT/ICES.

Alternatively, ASAT/ICES values could be assigned to primary or secondary (or both) ASAT reference materials in service laboratories by direct fundamental standardization with the IFCC Reference Method for ASAT at 29.772 °C. Few laboratories, however—even those with long-term research interests in enzyme standardization—would elect to go this route because of the time, effort, and expense involved. Likewise, few service laboratories will attempt to produce their own or even utilize other sources of the certified primary ASAT reference materials for similar reasons. However, once the reference system is in place, the costs of assigning ASAT/ICES values to the secondary calibrators will probably be less than is now the case, because the manufacturers of calibrators and quality-control materials will no longer need to assign 15 to 20 method-dependent values for each enzyme.

Secondary ASAT Reference Materials: NBS Human Serum SRM No. 909

In the last few years a lyophilized human serum reference material, the U.S. National Bureau of Standards' Standard Reference Material No. 909 (NBS/SRM No. 909), has been available to all clinical laboratories for calibration and quality control (82). This SRM carries certified concentration values for several nonenzyme analytes assigned by NBS scientists through use of Definitive Methods (88); however, the overall utility of NBS/SRM No. 909 to the clinical laboratory community, particularly in the development of multi-channel instruments, would be greatly enhanced if values for enzyme activity could also be assigned. Because the NBS Center for Analytical Chemistry had no staff scientist skilled in enzyme measurements, the ASAT catalytic activity concentration in this SRM was determined by a voluntary group of six cooperating scientists from five American laboratories, working cooperatively under the leadership of Dr. Robert Rej. Although staff scientists at NBS coordinated the study and provided statistical support, the final ASAT value is a consensus value obtained from data submitted by the six cooperating scientists, and it is not certified by NBS (60).

These investigators used the IFCC Reference Method for ASAT in assigning the ASAT value to NBS/SRM No. 909, but with three minor modifications, introduced as a result of the American ASAT Study Group's work (28). First, the reaction temperature set-point was dropped slightly from 30.00 °C to the most nearly accurately known reference temperature available to the clinical laboratory, the gallium melting-point at 29.772 °C (77). Secondly, the sample and reagent volumes were changed slightly to better fit the available volumetric glassware and pipettes. Third, no lactate dehydrogenase was used in the preincubation mixture.

This NBS/SRM No. 909 is perhaps the first enzyme reference material carrying an ASAT value assigned by the IFCC Reference Method that is available to any and all. Moreover, the exact methodological and procedural details of the method for ASAT finally used are unambiguously spelled out (60). Although NBS/SRM No. 909 is only a secondary ASAT reference material, it has served a unique role in helping us develop our concept of scale unification via ICES and the supporting ASAT reference system.

NBS/SRM No. 909 as an ASAT/ICES Calibration Standard

We have used this calibrated SRM material carrying ASAT/ICES values successfully to bring the ASAT/ICES scale to several different automated instruments of advanced design. However, before we accepted this material as an ASAT/ICES calibration standard, we first determined that we could still reproduce the original 29.77 °C values in this material, which by then was three years old, to well within the original stated uncertainty limits. We recovered slightly more activity than the 31 U/L originally assigned to this SRM (60), and this not only confirmed the stability claims but also verified the reliability of our analytical system.

We then measured the catalytic activity concentration of ASAT in human serum at 29.77 and 37.0 °C, using two recently introduced enzyme analyzers and NBS/SRM No. 909 as the calibration standard from which to calculate the serum activities. We were pleased to see that, with use of this stable secondary reference material, we were able to transfer the ASAT/ICES values equally well to both instruments at both temperatures, giving four nearly identical ASAT/ICES values for each serum sample!
Next, we used this SRM as the calibration standard in the enzyme workhorse of our laboratory, the Cobas-Bio centrifugal analyzer, at five temperatures (25.0, 29.77, 32.0, 37.0 and 40.0 °C) to determine ASAT activity in 20 patients' samples and in internal quality-control pools. Analyses of these samples in triplicate at each temperature setting yielded ASAT values ranging from 12 to 400 U/LICES. (The subscript signifies that the IFCC Reference Method for ASAT was used to determine the activity at the reaction temperature of 29.77 °C.) Statistical analysis showed the ASAT raw data for the 21 sets of results to have means and standard deviations that gave CVs ranging from 27 to 35%, averaging 31.1%. We then calculated the ASAT/ICES for the same 21 sets of results, using NBS/SRM No. 909 as the concurrent calibration standard, assigned a value of 31 U/LICES. The same statistical analysis of these results show that the CVs ranged from 1.5 to 12.5% (average 4.8%). The two average CVs (31.1% vs 4.8%) were statistically different (p = 0.005) by the F-test (83). Figure 3 shows a plot of results for five representative patients' samples and demonstrates the scale-unification power of ICES.

Application of the ICES Concept to Interlaboratory Survey Data

We have searched the literature on interlaboratory ASAT studies to see if the ICES concept could be used retrospectively to unify survey results. The following publications seemed particularly pertinent:

A. 1970 Scandinavian Survey. Strømme and Eldjarn (45)

Note in a survey of 116 Nordic laboratories testing two samples that the CV for enzymes (including ASAT) could in general be decreased significantly by use of one sample as a reference standard for the other. The average decrease in CV was from 36 to 16% for the enzymes tested.

B. 1971 New York State ASAT Survey. Rej et al. (39) sent out four stable ASAT primary erythrocyte reference materials in June 1971 and received results from 319 laboratories. Using the New York State Laboratories' ASAT values as the ASAT/ICES calibration values, we have reworked their data in Table 2. Note that the ASAT raw data for different methods gave CVs of 41 to 44% and the ASAT/ICES data gave CVs of 2 to 5%.

C. 1976 CDC Cooperative ASAT Experiment. The clinical chemistry group at the Centers for Disease Control (Atlanta, GA) prepared numerous batches of ASAT reference materials, following the method published by Rej et al. (36). Six specimens (R29-R34) were submitted to about 300 U.S. laboratories in 1967, and 283 sets of results for ASAT were returned. The ASAT raw data for R30 ranged from 6 to 67 U/L with a mean and standard deviation of 29.0 ± 9.8 U/L for a CV of 33.9%. Six reference laboratories used the IFCC Reference Method for ASAT (32) to assign the catalytic activity concentration value of 58 U/L for R31, and we used this activity in the ASAT/ICES calculations. After dividing R30 by R31 and multiplying by 58 U/L, the ASAT/ICES data for R30 among all reporting laboratories ranged from 18 to 41 U/LICES, with a mean and standard deviation of 30.0 ± 3.2 U/LICES and a CV of 10.8% (the six Reference Laboratories' value for R30 at 29.77 °C was 29.9 U/LICES).

D. 1978-1982 Dutch Studies. The efforts of the Netherlands Society for Clinical Chemistry to standardize enzyme activity measurements by use of nationally recommended enzyme methods and enzyme standards have been described by Jansen and Jansen (63). ASAT results from 40 laboratories, which were using all types of routine methods with reaction temperatures ranging from 25 to 37 °C, gave a CV of 50%—which decreased to 15% with use of the recommended method (31) and to 10% when both the recommended method and an enzyme standard were used.

E. 1983 CAP Comprehensive Survey. The ASAT activity in sample C-7 (cited earlier) and in C-8 were measured by about 4000 U.S. clinical laboratories and the results were reported as the mean, standard deviation, and CV for 36 distinct method groupings (1). Our clinical enzyme laboratory measured the activity in samples C-7 and C-8 by the IFCC Reference Method for ASAT at 29.77 °C and obtained values of 85 and 87 U/L, respectively. The mean values for the ASAT raw data of C-7 ranged from 61.5 to 152.2 U/L, with an overall mean and standard deviation for the 36 method means of 93.1 ± 31.4 U/L and a CV of 34.7%. After ASAT/ICES conversion by ratioing C-7 to C-8 and multiplying by the ASAT/ICES C-8 value of 87 U/LICES, the range narrowed to 82 to 87 U/LICES, the overall mean, and standard deviation for all 36 methods was 93.1 ± 55 U/LICES, and the CV was 0.66% (Figure 4).

F. 1983 CAP Enzyme Chemistry Survey. The ASAT activity in sample V-05 in this 1983 CAP interlaboratory enzyme survey was ratioed to sample V-04 for the 19 ASAT method groupings (1). The overall combined method CV of 33.3% for the ASAT raw data for V-05 alone decreased to 2.1% by this ratio technique.

The Potential Unification of ASAT Values Assigned to Quality-Control Materials

The critical decision that manufacturers of calibration and control materials face is what ASAT method to use to assign the value to a stable ASAT preparation. Since no one
Table 2. 1971 NY State Laboratory ASAT Survey of 319 Labs

<table>
<thead>
<tr>
<th>Method, units, temp.</th>
<th>Reported (U/L)</th>
<th>Calculated from C (U/LICES)</th>
<th>Reported (U/L)</th>
<th>Calculated from C (U/LICES)</th>
<th>Reported (U/L)</th>
<th>Assigned (U/LICES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYS value (assigned)</td>
<td>17</td>
<td>17</td>
<td>46</td>
<td>46</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Reference lab U/L, 30 °C (kinetic)</td>
<td>18</td>
<td>18</td>
<td>48</td>
<td>48</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Karmen conditions, U/L, 30 °C</td>
<td>14</td>
<td>17.6</td>
<td>38</td>
<td>47.8</td>
<td>74</td>
<td>93</td>
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<tr>
<td>Eskalab U/L, 37 °C</td>
<td>24</td>
<td>16.9</td>
<td>68</td>
<td>47.9</td>
<td>132</td>
<td>93</td>
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<td>Sigma colorimetric Reitman–Frankel units, 37 °C</td>
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<td>15.9</td>
<td>68</td>
<td>49.0</td>
<td>129</td>
<td>93</td>
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<tr>
<td>Dade colorimetric, Reitman–Frankel units, 37 °C</td>
<td>33</td>
<td>19.3</td>
<td>82</td>
<td>48.0</td>
<td>159</td>
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<td>Geni. Diagnostics colorimetric</td>
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<td>117</td>
<td>47.5</td>
<td>229</td>
<td>93</td>
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<tr>
<td>TransAc., 37 °C</td>
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<td>18.5</td>
<td>141</td>
<td>50.2</td>
<td>261</td>
<td>93</td>
</tr>
<tr>
<td>SMA 12/60 calorimetric U/L, 45 °C</td>
<td>27.9</td>
<td>17.6</td>
<td>76.3</td>
<td>48.1</td>
<td>146.3</td>
<td>93</td>
</tr>
<tr>
<td>Mean</td>
<td>13.6</td>
<td>±1.0</td>
<td>±36.2</td>
<td>±1.2</td>
<td>±67.1</td>
<td>—</td>
</tr>
<tr>
<td>SD</td>
<td>45.6</td>
<td>6.8</td>
<td>42.5</td>
<td>1.8</td>
<td>41.3</td>
<td>—</td>
</tr>
<tr>
<td>CV, %</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Reji et al. (39).

"Authoritative" method exists, the manufacturer assigns to the sample multiple activity values for ASAT, each corresponding to a particular method in current use. For example, the package insert accompanying the Technicon chemistry control material called TQC ALERT 2 provides the purchaser with 13 ASAT values ranging from 82 to 256 U/L (84). Similar numbers of methods and a wide range of activities can be found on other commercial reference materials designed for quality control and calibration.

When we applied the ASAT/ICES concept to the multilevel information given in the package insert of Beckman's decision material (85) and assigned ASAT/ICES values of 25, 40, and 76 U/LICES to the control materials (Level 1, 2, and 3, respectively), the overall combined CVs for ASAT declined from 25.6% and 19.8% for the raw data of Level 1 and 3 down to ASAT/ICES data of 3.6% for both when the Level 2 material was used as an internal calibration standard. We anticipate that incorporation of the ASAT/ICES concept into the commercial calibrator/quality control system with only a single ASAT/ICES value assignment will lead to considerable simplification of work and associated dollar savings for all. More importantly, we would expect major improvements in the interlaboratory compatibility of results for ASAT.

Discussion

This proposal for ASAT/ICES had its origin in a decade of activity within the EPE/IFCC that included lengthy and repeated discussions on the role of both enzyme reference methods and enzyme reference materials. These EPE/IFCC efforts have resulted in carefully documented, detailed recommendations for the following: Part 1—General Considerations for Reference Methods (33), Part 2—Method for Aspartate Aminotransferase (32), Part 3—Method for Alanine Aminotransferase (85), Part 4—Method for Gamma-Glutamyltransferase (87), Part 5—Method for Alkaline Phosphatase (88), Part 6—General Considerations for Enzyme Reference Materials (66), Part 7—Method for Creatine Kinase (89), Part 8—Method for Amylase (proposed for study in 1984) (90), Part 9—Method for Lactate Dehydrogenase (proposed for study in 1984) (90). These important EPE/IFCC documents and the many national recommendations on enzymes clearly reflect the fact that our cooperative national and international standardization efforts in clinical enzymology were directed almost exclusively at reference method technology. The need to have and to use stable, well-characterized enzyme materials to develop better enzyme reference methods has not been totally ignored (21, 32, 48, 53), particularly as evidenced by the purification and use of ASAT material from human erythrocytes (36) and from other organs from different species (58). However, in spite of the increasingly successful use of stable enzyme prepara-
tions in calibrators (51, 56, 62), in control sera (46, 54–56), and in survey materials (36–39, 47, 49, 63–65) over the past two decades, ill-defined myths concerning sources, stability, isoenzymes, characterization, costs, and availability of primary human enzyme reference materials seems to have prevented their systematic incorporation into our concepts of enzyme standardization until very recently (2, 36–40, 63).

The EPE/IFCC reference material efforts have only recently surfaced as a first internal draft document (Part 6) (68) that now takes its place alongside the other IFCC enzyme reference recommendations (61). The insight about the unifying power of enzyme reference materials that Strømme and Eldjarn (45) wrote about in 1970, saying "In our opinion the only possible avenue of approach at present appears to be that all laboratories agree upon the use of one enzyme solution as reference standard against which the various methods should be calibrated," has since been shared by and added to by many other investigators (2, 47, 49, 50, 52, 53, 57, 61–63, 65). These publications, taken together, speak clearly to the need for an increased emphasis on the use of stable enzyme reference materials. For example, the general conclusion from the Dutch studies reported by Jansen and Jansen (63) is one that unequivocally supports the incorporation of stable enzyme calibration standards into future enzyme-standardization schemes. Our view is that enzyme standardization must rest upon a hierarchical reference system that at every level fully exploits the interdependency of sound enzyme methods and stable enzyme materials. Fortunately, many individuals and groups have been or are now hard at work to develop these much-needed stable, human-origin enzyme reference materials, including the following: acid phosphatase (91), alkaline phosphatase (92), amylose (93), creatine kinase (94, 95), γ-glutamyltransferase (96), and lactate dehydrogenase (97).

Unfortunately, there exists no international "enzyme authority" that has been given widespread support to create and maintain the agreements required for a functional unifying reference system for enzymes. Some may argue that the EPE/IFCC has been given this charge by the executive committee and officers of the IFCC; however, the "enzyme authority" of the EPE/IFCC has been challenged and perhaps even seriously undermined by the IFCC's own Expert Panel on Instrumentation, which has recommended the universal use of only 37 °C for enzyme measurements (35). Fortunately, the resolution of this conflict over reaction temperature is technically quite simple within the framework of a hierarchical ASAT reference system approach.

For many reasons, ASAT is an ideal enzyme with which to develop the formal agreements concerning the global aspects of reference systems for clinical enzymology. First, the national and international recommendations for ASAT reference methods are very similar, as shown in Table 1. Second, stable primary ASAT reference materials of human origin have been produced by several independent groups (96, 37, 58). These have been carefully studied as transfer materials (38, 39) and have been accepted for their suitability as primary ASAT reference materials by the NRSC Council (40). Third, many other stable human and animal source ASAT secondary reference materials, calibrators, and quality-control materials have been compared to these primary ASAT reference materials and have been shown to perform well in long-term daily use in working laboratories and in surveys (e.g., 1, 60, 63, 64). Fourth, as shown by investigations with several primary and secondary ASAT materials and also with patients' sera, reaction temperature changes between 25 and 37 °C cause little or no difference in the final reagent concentrations of key reagents (24, 36, 37, 58). Fifth, the evidence from our experiments (see Figure 3) and from ASAT/ICES conversion of ASAT data taken from interlaboratory surveys unequivocally demonstrates that formerly incompatible numerical values for ASAT results are readily made compatible by conversion to the ASAT/ICES scale.

The time has come to provide compatible numerical enzyme results from all laboratories. The reference-system approach and the ICES proposal give the clinical laboratory and medical community a universal means of creating and ensuring the compatibility of enzyme measurements. As shown here with a model for ASAT, the use of a hierarchy of stable reference, calibrator, and control materials carrying ICES values makes possible the direct calibration of any other working method at any other temperature at any loci to the one unifying scale of ICES. Scale unification to ASAT/ICES by use of calibrated enzyme reference materials carrying the ASAT/ICES value of the IFCC Reference Method for ASAT at 29.77 °C to all loci gives us the means to have a truly universal, inclusive reference system based upon state-of-the-art reference technology without requiring disruptive changes in the existing working methods or in an individual's preference for one or another reaction temperature.

As documented above, the necessary technical items are now at hand to construct an ASAT reference system. We must now choose either to continue on our independent ways, producing sound but incompatible results, or to create the international agreements needed to bring about compatibility through scale unification. The voluntary consensus process concerning these state-of-the-art ASAT reference method and ASAT reference materials is now underway in the clinical laboratory community of the U.S. If this widespread NCCLS review is favorable, these specific proposals regarding ASAT reference materials and ASAT reference methods will be the key items of the ASAT reference system. Improved interlabatory compatibility of ASAT results due to the ICES unifying concept should thus become a reality in the U.S. in the near future.

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