that the manual kit and the saccharogenetic procedure "correlated quite well."

Melnychuk (6) reported an evaluation of four commercially available amylase tests as well as their own saccharogenetic method. The standard or reference method had a coefficient of variation of 22.7%, while all the commercially prepared reagents had a coefficient of variation that was well under 10%. We do not imply that the test that has the smallest coefficient of variation gives the correct result, but how can one possibly find the correct result when using methods that give such wide variability? This definitely represents a classic example where both precision and accuracy have to be considered.

This same paper gives data on 20 normal subjects and 40 in-patients. If the saccharogenetic method is assumed to give the correct result on all patients then the Phadebas amylase test is the only method of those reported that gives values on patients that agree with the clinically accepted normal and abnormal amylase values.

Phadebas amylase substrate is prepared in large batches. Reagents from single batches are usually available for at least a year. We strongly recommend that laboratories prepare and preserve (or purchase) a supply of reference sera that they can calibrate for use as a reference material for the calibration of the Phadebas amylase test. Calibration curves that have been prepared on a Ziehl PMQ II by using amylase from human sources are available on request for those who wish to request them.

References

R. Ali

Department of Biological and Clinical Research
Pharmacia Laboratories Inc.
800 Centennial Ave.
Piscataway, N. J. 08854

Standards in Clinical Chemistry

To the Editor:
Dr. Meites is absolutely correct in stating [Clin. Chem. 19, 789 (1973), letter] that procedures in clinical chemistry should be standardized with the purest standards available in the most definable solutions possible. This is a point of view with which one cannot argue. Several publications have discussed this issue (1–3). However, it ignores several significant facts.

Fact 1: Practicality. With the proliferation of multichannel analyzers, it is impractical to standardize one channel at a time, several times an hour. This difficulty can be overcome by using a multicomponent standard prepared from the purest substances available, in the most definable solution possible. Since total protein, no doubt, will be one of the quantitative procedures of interest, a protein should be included in the standard. Human serum albumin might be considered a source of protein, although this substance would certainly be ill-defined by pure analytical chemical standards.

Fact 2: Availability. As stated by Dr. Meites, in some cases and notably with respect to enzymes, pure sample of standards are not available commercially. Moreover, there seems to be no agreement as to what the pure standard would be. For example, alkaline phosphatase of human origin preferred to a nonhuman source of this enzyme? If so, which isoenzyme should be used? The actual test procedure will assay all isoenzymes without differentiation. This difficulty can be overcome by using the pure end-product of the reaction to calibrate the detection device. The danger of this technique, however, lies in the fact that sub-optimal substrate concentrations and the presence of enzyme inhibitors will remain undetected and uncorrected. Another problem with this technique is the difficulty of obtaining such end-products in sufficient purity for calibration. One notable example of this problem is oxalactic acid that is required for the standardization of aspartate aminotransferase. We have examined samples of oxalactic acid that varied in purity from 50% to 90%, even though all samples were represented as being 95% pure.

Fact 3: Interferences. In many instances, analytical procedures are affected by the presence of protein, especially if dialysis is a part of the method. In these cases, pure aqueous standards give a response that differs from that obtained with protein-containing samples, including human serum, and leads to erroneous results. Owing to their insolubility in water, some pure standards must be made into solutions that may alter the constituent in such a manner as to render the quantitative preparation of these standards unreliable by the average laboratory. Bilirubin and cholesterol are examples. These difficulties usually are overcome by the use of secondary standards that have been assayed against primary standards and methods that are unaffected by these difficulties.

At Technicon, we fully recognize the deficiencies encountered in the use of secondary standards but we are also cognizant of the needs of the average laboratory. All of the values assigned to our products ultimately refer back to primary standards whenever practical and available, and our literature reflects this fact. A recent publication by Schneider and Cornette (4) fully discusses this approach to the problem.

References

Paul Schneider
Director, Technical Development and Evaluation
Technicon Instruments Corp.
Tarrytown, N.Y. 10591

Commercial Immunodiffusion Plates

To the Editor:
After reading the article by Hosty et al. (1) and evaluating the data presented, it was evident that

1. Sampling of specimens was inadequate to reach any definitive conclusion. (This was freely admitted by the authors in the latter portion of the article.)
2. The authors neglected to mention how many replicate studies were made to determine the value of the International Reference Serum (batch 95/69) and the 12 individual sera tested.
3. The range of values obtained with companies' references and plates by the capillary technique shows merely that either filling or technique, or both, was inadequate. The manner in which these results were obtained was not indicated, apparently only one of three standard references provided were measured.

Commercial Immunodiffusion Plates

To the Editor:
After reading the article by Hosty et al. (1) and evaluating the data presented, it was evident that

1. Sampling of specimens was inadequate to reach any definitive conclusion. (This was freely admitted by the authors in the latter portion of the article.)
2. The authors neglected to mention how many replicate studies were made to determine the value of the International Reference Serum (batch 95/69) and the 12 individual sera tested.
3. The range of values obtained with companies' references and plates by the capillary technique shows merely that either filling or technique, or both, was inadequate. The manner in which these results were obtained was not indicated, apparently only one of three standard references provided were measured.