4. Pharmacia Research Group fully evaluates the rationale and practicability of all new and suggested modifications to an established procedure such as the Alpha-Amylase test before it is finally recommended in our package insert. The effect of adding bovine serum albumin or PVP to the incubation mixture is currently the subject of intensive investigation in our research organization. Our findings on this subject will soon be ready for release.

In the meantime, we appreciate the suggestion by Dr. Berger and his associates and all other concerned groups to add bovine serum albumin or PVP to our reagents mixture for urinary amylase. However, similar studies are also needed on other available α-amylase methods in order to establish the merits of proposing this additional step.

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Faster Continuous-flow Analysis for Triglycerides and Cholesterol

To the Editor:

Increasing requests for triglycerides (TG) and cholesterol (CHOL) reflect the recognized diagnostic importance of cardiovascular risk factors. For this reason our laboratory has changed during the last two years from manual performance of these two tests to a partly mechanized simultaneous method on the AutoAnalyzer II. CHOL is determined according to Technicon Method No. 24a and TG are fluorometrically determined after Kessler and Lederer (1). With this original method we have a CV of 3.79% day to day and 1.44% within a series for CHOL. A CV of 3.41% from day to day and 2.35% within a series was found for TG. Furthermore we are no longer satisfied with a total of 50 tests per hour, which is recommended by Technicon in the method description. Use of the 50/1 cam is recommended by Technicon to avoid carryover. To increase the test rate we used the 80/1 cam instead the 50/1 cam and found no appreciable carryover. The CHOL-channel was left completely as in the original Technicon description. The heating-bath manifold, pump configuration, and fluorometer remain the same for the TG-channel in our 80/8 method as in the 50/8 method. The only modifications are simple, between the metal sample needle and the pump. Technicon recommends a Kel-F probe connection for TG to be not longer than 51 cm. We used in place of the Kel-F for a connection an orange-yellow pump tube (0.02" i.d.) of Solvaflex, cut to a length of 20 cm. The thin metal needle can be directly inserted into one end of this 20-cm Solvaflex connecting tubing; the other end is connected to the pump by a metal nipple. We found excellent correlation between results by the Technicon original procedure and our modifications. Our modifications have two advantages:

1. Quality of the tests is identical and carryover is not greater.
2. The modifications are simple and inexpensive. We can therefore save reagents and at the same time run 1.5-fold as many tests.

Reference


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Ed. note: These authors supplied documentation, which is available on request from the Editorial Office or from the authors.

A Lipase Problem in an Enzymatic Procedure for Serum Triglycerides

To the Editor:

We recently described a single reagent enzymatic procedure for serum triglyceride determination [Clin. Chem. 21, 1983 (1975)] which may be used with the Abbott Bichromatic Analyzer (Abbott Labs., Diagnostics Division, Pasadena, Calif. 91030). The reagent was prepared by combining the contents of the Triglyceride-Glycerol (3-vial) reagent (Calbiochem, La Jolla, Calif. 92037) into one reagent.

Recently we received a new lot number of triglyceride reagent and found the method did not perform as we described. Investigation revealed that via C (lipase) did not display the activity in the combined reagent we reported previously. Instead of quantitatively hydrolyzing the triglycerides in serum samples with elevated values in less than 4 min, 10 to 15 min was required. The same reagents appeared to work satisfactorily in the Abbott recommended procedure, because the lipase is incubated with the sample 30 min prior to the final reading. We therefore caution laboratories using this procedure to compare the results obtained on several control sera and patients' samples against the Abbott recommended procedure on the ABA-100 with each new lot number of Calbiochem triglyceride reagent.

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Effect of Glutamate on γ-Glutamyltransferase Activity

To the Editor:

Bonder and Moss (1) produced data based on two patients' sera that showed a stimulation of γ-glutamyltransferase activity (γGT, EC 2.3.2.2) in the presence of glutamate. Rock and Slickers (2) attempted to confirm the findings of Bonder and Moss, using nine patients' sera of normal and abnormal activity, assayed in triplicate, and found no activation of γGT such as was originally indicated by Bonder and Moss, either in any individual specimen or in the nine sera collectively.

In reply to the work of Rock and Slickers, Bonder and Moss (3) repeated their initial experiment on four additional sera and again demonstrated an increase in γGT activity in all sera within a range of glutamate concentration 60-80 mmol/liter. They could not repeat their initial claim of 100% activation but showed an average activation of 44%. The activation peak at 1 mmol/liter concentration of glutamate, described in their original paper, could not be confirmed. Bonder and Moss also used Student's t-test to reevaluate the data presented by Rock and Slickers and suggested that an activation was actually present.

In addition, a recent paper by Gerhardt et al. (4) has shown that no increase in γGT activity could be demonstrated when L(+)-glutamate (0.01 to 5.0 mmol/liter) was incorporated into the reaction media and the assay done at 25, 30, or 37 °C.

In the light of the conflicting situation regarding activation of γGT by L-glutamate, we attempted to repeat the work of Rock and Slickers and also to ascertain the effect of L-glutamate on the γGT assay currently used in this laboratory.

We selected ten patients' sera of various γGT activity to be assayed in triplicate by two methods in the pres-
ence of increasing concentrations of L-glutamate. The final reaction mixture for the first method approximates the method used by Rock and Slickers, but was done at 37 °C and contained, per liter, 4 mmol of γ-glutamyl-p-nitroanilide (Sigma), 40 mmol of glycylglycine, and 185 mmol of tris (hydroxymethyl) aminomethane buffer (pH 8.25). L-Glutamic acid (Sigma) was added to the buffer to give a final concentration in the reaction mixture ranging from 0 to 1.0 mmol/liter. The final reaction mixture had a sample/volume ratio of 1/29. The reaction was measured by measuring the rate of change of absorbance at 410 nm at 37 °C, by use of an LKB Model 8600 reaction rate analyzer, and the activity was calculated in U/liter.

The final reaction mixture for the second method (5)—a method currently in use in this laboratory—contained, per liter, 8 mmol of γ glutamyl-p-nitroanilide (Sigma), 67 mmol of glycylglycine, and 100 mmol of 2-amino-2-methylpropane-1,3-diol buffer (pH 8.30). The final reaction mixture had a sample/volume ratio of 1/12. Identical concentrations of L-glutamate were used as in the first method.

Data collected during this study showed good precision, similar to that described previously (5), and a similar precision for both the first and second methods.

Table 1 shows the mean results of triplicate determinations. The assays were performed in the order shown left to right in the Table, each set of results for a given patient being obtained within the same analytical batch. A zero concentration of L-glutamate was analyzed at the beginning and end of each patient batch. These results are reported to one decimal place, but one should not infer that single γGT determinations are this precise. The precision of these results is about 0.5 to 1.0 standard deviation units as a result of their being the mean of triplicate determinations.

Comparison of activities with Student's t-test for paired observations did not show any significant activation (P > 0.1) by either of the two methods at any concentration of added glutamate. The first method showed a decrease in γGT activity at 0.5 mmol/liter concentration of L-glutamate (0.05 < P > 0.02) and a highly significant (P < 0.001) decrease at 1.0 mmol/liter L-glutamate concentration. This is possibly the result of product inhibition at the lower (and suboptimal) substrate concentration used in this method.

References
5. Farrance, J., Krauja, V. W., and Dennis, P. M., The determination of γ-glutamyltranspeptidase by reaction rate assay at 37 °C. Pathology 7, 257 (1975).

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Bichromatic Analyses with the ABA-100—A Critical Assumption

To the Editor:

A bichromatic analyzer measures the difference in absorbance at two preselected wavelengths (A2 = Aλ1 − Aλ2). An underlying assumption is that the absorbance at the blanking wavelength (λ2) relative to the test wavelength (λ1) is the same for all standards and samples. Said another way, if standards and samples were appropriately diluted, their absorbance spectra would be superimposable. If a sample absorbed relatively more at the blank wavelength than at the test wavelength, a falsely low result would be obtained. Conversely, if a particular sample absorbed relatively more at the test wavelength than at the blank wavelength, a falsely high result would be obtained.

We evaluated the HyCel procedure for serum (and plasma) phosphorus measurements on the ABA-100 bichromatic analyzer (Abbott Scientific Products Division, South Pasadena, Calif. 81030). During the first month of running the ABA-100 procedure in parallel with our routine procedure (Technicon N-39 on an AutoAnalyzer I system), we found that about 4% of the samples (8 of 184) gave results that differed by more than 10 mg/liter. A few months later, when we were running in parallel again as part of a program to train medical technol.