letters

Noise Reduction in an AutoAnalyzer II Direct Cholesterol Method

To the Editor:

Output peak distortion and noise are a problem in the AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, N. Y. 10591) direct cholesterol procedure no. SE4-0026 FC4. We originally believed this difficulty to be inherent in the two-step sampling procedure used in this method, but we later observed sample diluent back-up into the bubbler tube of the original injector block, apparently because of the relatively large cross-sectional area and the resulting dead-space volume of the bubbler tube. This effect may have been increased by the relatively large sample volume (0.4 ml) introduced. The fluid level in the bubbler tube varied continually. Hence sample slug length and concentration varied, producing considerable noise and abnormally shaped output curves.

The following modifications considerably reduce noise and improve the output curve shape in this procedure.

(*a*) Replace the sample injector fitting no. 116-0490-01 with injector fitting no. 177-B004-02, which has a considerably smaller cross-sectional area and interacts little with stream flow, except during bubbling.

(*b*) Replace the original sample diluent (distilled water) with a 2 g/liter solution of the surfactant Brij-35, which improved bubble pattern.

(*c*) Replace the pump tube feeding the sample injector bubbler so as to decrease the flow rate from 0.42 ml/min (no. 116-0549-08 tubing) to 0.32 ml/min (no. 116-0549-07 tubing), to limit surging.

(*d*) Rotate the *A10* (no. 116-B034-01) sample fitting from parallel to upright position.

These modifications have significantly decreased both the coefficients of variation as well as the frequency of need to repeat assays. Three controls, a borderline low, borderline high, and normal were used for two months before the modification and two months afterwards. The coefficients of variation before the modification were 2.8, 3.7, and 3.1%, respectively, as compared with 1.6, 2.5, and 1.1% afterward. The probability (*P*) that these improvements in precision occurred by chance are less than 0.01, 0.05, and 0.001, respectively (*F* test). Student's *t*-test showed no significant difference in the means at any of the three analyte concentrations before and after modification.

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Inaccuracy Detected in Acid Phosphatase Kit Method

To the Editor:

Coulter Reagents, Inc. (Maunabo, Puerto Rico) manufactures a test package for the manual analysis of serum acid phosphatase, which is distributed by Coulter Electronics, Inc., Hialeah, Fla. 33014 and marketed in the United States as "C-Zyme Acid Phosphatase." This kit method represents an adaptation of the original test procedure of Roy et al. (*1*); sodium thymolphthalein monophosphate is used as substrate in a reaction conducted at pH 6.0. Addition of alkali terminates enzyme hydrolysis and, at the same time, converts the liberated thymolphthalein to a colored complex.

According to the insert procedure sheet (*2*) accompanying the Coulter test package, 0.2 ml of sample (test, controls, and standard) is added to 1.0 ml of "C-Zyme Acid Phosphatase Reagent" (a freeze-dried reagent which, on reconstitution with distilled water, provides 2.2 mmol of sodium thymolphthalein monophosphate, 100 mmol of citrate buffer, and 5.5 g of "surfactant" per liter), and the resulting mixture is incubated for 30 min at 37 °C. Afterwards, 5.0 ml of "Color Developer" (100 mmol of sodium carbonate and 100 mmol of sodium hydroxide per liter) is added, and the absorbance of the final mixture is measured vs. a reagent blank set at zero absorbance at 595 nm. Calibration of the test is based on a thymolphthalein standard (1.0 U/liter) supplied in the test package along with all other reagents required for analysis.

When analyzed as a test sample by the Coulter kit method (all test-package lots studied including lot no. 6501), a 10.0 U/liter thymolphthalein standard (12.9 mg of thymolphthalein, obtained from Eastman Kodak Company, Rochester, N. Y., dissolved in sufficient ethanol to make 100 ml of solution) produced results that were about *half* as great as expected (4.9, 5.4, 4.6 U/liter are typical results in between-day, between-technician testing). The kit method was performed without modification according to the exact procedure specified by the manufacturer.

According to Coulter Diagnostics, Inc. (*3*), the source of the inaccuracy (the "1.0 U/liter" standard supplied in the test package was found to be 2.0 U/liter) has existed since the test kit package was introduced commercially sometime "before 1975." Although the manufacturer cannot be without responsibility for the error, the fact that such an inaccuracy has gone undetected for over two years at a national and possibly international level suggests that the precaution of carefully evaluating commercially supplied standards is not taken seriously by users.

This example, as well as one reported earlier (*4*), emphasizes the need for careful user evaluation of commercial test products.

References

1. Roy, A. V., Brower, M. E., and Hayden, J. E., Sodium thymolphthalein monophosphate: A new acid phosphatase substrate with greater specificity for the prostatic enzyme in serum. *Clin. Chem.* **17,** 1093 (1971).

2. Coulter C-Zyme Acid Phosphatase Test Package insert document No. 4202168, issued 2/75. Coulter Electronics, Inc., Hialeah, Fla.

3. Pu, G. (Manager, Chemistry Systems Liason, Coulter Diagnostics, Inc., Hialeah, Fla.), personal communication.

4. Bogdan, D. P., and Bishop, C. W., Differences between manufacturer-assigned values and observed values of alkaline phosphatase activity in a commercial SMA calibration serum. *Clin. Chem.* **20,** 1244 (1974).

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A representative of Coulter Diagnostics responds:

To the Editor:

We are grateful to Dr. Bogdan for his observation and prompt report to us. We have confirmed Dr. Bogdan's findings that the 1 U/liter thymolphthalein standard which we provide with our serum acid phosphatase reagent is actually equivalent to 2 U/liter acid