capillary, the nine negative specimens were sampled and processed. This experiment was repeated with a plastic capillary.

With the glass capillary, the first specimen was positive as expected, but the next eight specimens were also positive. The last specimen was negative. For the plastic capillary, the first specimen was positive and the next three originally negative specimens were now positive. The last six negative specimens were still negative. These experiments clearly demonstrate that carryover of HBAg is significant enough to give an erroneous result on a number of successive specimens, depending upon the type of capillary used.

We did another experiment to determine how feasible it would be to wash the capillary with several water rinses before sampling the next specimen. Even though our first experiment indicated that counts were reduced to background after six washes with glass capillaries and after three washes with plastic capillaries, as many as 10 rinses, in some cases, did not prevent a negative specimen following a positive specimen from also being positive. Twenty washes did, however, eliminate the problem.

In HBAg testing it is not practicable to wash 20 times between each specimen; the best solution is to use a new tip or capillary for each specimen.

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Thymol Is a Suitable Preservative for Uric Acid Standards in the Uricase Technique

To the Editor:
Preservatives such as formaldehyde, sodium azide, and thymol in standards interfere with the quantitation of serum or urinary uric acid by various colorimetric procedures such as phosphotungstic acid reduction and direct acid ferric reduction procedures (1-4). However, it is generally accepted that the uricase method is more specific and free of these interferences exerted by the reductive compounds commonly affecting the colorimetric procedures. Because of its specificity, we implemented an uricase method for serum and urinary uric acid for use with a centrifugal analyzer (Union Carbide). Standards must contain a preservative. Formaldehyde is excluded as a possible preservative because it inhibits uricase (5), sodium azide because its use involves health and safety hazards (6, 7). I have shown that thymol is suitable for this purpose. I first prepared two sets of aqueous standards, one of them in saturated thymol solution. I added 50 μL each of the standard to 3.0 mL of borate buffer (0.2 mol/L, pH 9.5). Ten minutes after adding 20 μL of uricase (1 g/L, Boehringer Mannheim Biochemicals, New York, NY 10017), I measured the decrease in absorbance at 292 nm caused by the uricase-catalyzed hydrolysis of uric acid. On plotting the relation between the decrease in absorbance at 292 nm and concentration of standards in the presence and absence of thymol, I found it to be linear and the same whether or not thymol was present (Figure 1). This same observation held true when the analyzer was substituted for the manual procedure. Moreover, with thymol-preserved standards for calibration, values for serum uric acid of 121 patients, determined by the uricase method, correlated excellently with those by a continuous-flow method that is based on phosphotungstic acid reduction (correlation coefficient = 0.99). Standards in saturated thymol solution were stable for at least 14 weeks.

Because thymol exerts no effect on uricase activity and is a suitable preservative, I recommend that it be used for standards if quantitation is performed by the uricase method.

References
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Simplified Enzymic/Colorimetric Serum Urea Nitrogen Determination

To the Editor:
It is possible to simplify the enzymic colorimetric method (urease-Berthelot) for determination of serum urea nitrogen by combining urease (EC 3.5.1.5), nitroprusside, and salicylate into a single "enzymic reagent" and using sodium hydroxide and sodium hypochlorite as a second "color reagent." Thus only two reagents are needed instead of four (1), or three (2,3).

The enzymic reagent contains, per liter, EDTA 1.34 mmol, urease 50 000 U, sodium salicylate 62.45 mmol, and sodium nitroprusside 3.36 mmol. The final pH (at 25 °C) is 5.8 ± 0.1. The reagent is stable for 15 days at 4 °C. The color reagent contains sodium hypochlorite, 7 mmol/L, and sodium hydroxide, 150 mmol/L. It is stable for three months at 4 °C.

Procedure: Mix 20 μL of serum or plasma (collected without fluoride or ammonium salts of the usual anticoagulants) with 2.5 mL of the enzymic reagent; after 5 min at 25 °C (or 3 min at 37 °C) add 2.5 mL of the color reagent. After 10 min at 25 °C (or 5 min at 37 °C) read at 600 nm vs. a reagent blank. The final color is stable for at least 2 h.

The absorbancies are converted into serum urea nitrogen concentrations by means of a calibration curve (which we find to be linear up to 100 mmol/L) or by means of a calculation from a single serum urea nitrogen standard; 0.001 A corresponds to 50 mmol of serum urea nitrogen per liter.

Within-run precision (CV) was 0.6 and 0.7% for serum urea nitrogen concentrations of 10 and 41 mmol/L, respectively. Between-run precision (CV) was 1.7 and 0.6% at serum urea nitrogen concentrations of 15 and 60 mmol/L, respectively. The average analytical recovery of serum urea nitrogen added to
a pool of normal human sera was 99.2% ± 0.4%, linear between 6.5 and 20.0 mmol of added serum urea nitrogen per liter.

We compared this method with the Chaney-Marchbach method (2) and with a uv-kinetic method (4), with the following results: y = 1.00x - 0.08; r = 0.993 (n = 30), and y = 0.94x + 0.94; r = 0.990 (n = 26).

References

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Least-Squares Evaluation of Linearity

To the Editor:
The advent of the inexpensive pocket calculator, with hard-wired least-squares programming, has greatly increased the use of this numerical method. The chief drawback of the procedure is that it will give a slope and intercept for any set of numbers, even though they do not follow a linear equation of the form

\[ y = mx + b \]  

(1)

where \( y \) is the value of the dependent variable, such as absorbance, \( x \) is the value of the independent variable, such as concentration, and \( m \) (slope) and \( b \) (intercept) are constants. A valuable and detailed study of the use of this form of equation in the method of least-squares was published recently (1).

Though a graph can be prepared and inspected visually, I suggest here an alternative, entirely numerical method. After the determination of \( b \) and \( m \) by the method of least-squares, the equation

\[ y - b = w = cx^a \]  

(2)

is written, in which the new constants, \( c \) and \( n \), are introduced. This equation is then written in logarithmic form

\[ \log w = n \log x + \log c \]  

(3)

Equation 3 can be readily handled by the method of least-squares, with log \( w \) as the dependent variable and log \( x \) as the independent variable, to enable the constants \( n \) and \( c \) to be determined. If the system is truly linear, it is expected that \( n \) will be found to be equal to unity and that \( c \) will be found to be equal to \( m \). That is, for true linearity, \( n = 1 \) and \( c = m \).

Equation 3 exists only for testing purposes and I do not imply that it fully describes any empirical or otherwise real situation. It will, however, describe at least part of a curvilinear relationship, should one exist. It is seen that equation 1 is a special case of equation 3, when \( n = 1 \).

The use of this method requires two consecutive least-squares calculations. It is sufficiently simple that it can easily be put into a computer. A positive value of \( (n - 1) \) means that the curve is concave upward and a negative value of \( (n - 1) \) means that the curve is concave downward. I believe that stating the value of either \( n \) or \( (n - 1) \) in colorimetry will be a useful index of how well a system either follows Beer's law or deviates from it. This should be of use to clinical analysts in choosing or otherwise evaluating current or new methodology. In some instances it may be of value to determine \( n \) or \( (n - 1) \) over a limited range and (or) to set limits over how much \( (n - 1) \) may deviate from zero. This last point may have utility for purposes of quality control. It may be programmed into a computer for the purpose of validating the calibration.

Reference

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More on the Detection of Serum CK-BB Activity and Nonspecific Fluorescence

To the Editor:
The importance of CK-MB assay in the diagnosis of acute myocardial infarction is well established (1-3). In a normal serum, CK-MM predominates and CK-BB (or CK1) is not ordinarily observed by use of present methods. Most electrophoretic methods for separating and identifying CK isoenzymes localize enzyme activity, either in a cellulose acetate membrane or an agarose gel used in the separation. The Rosalki reagent formulation (4) allows one to measure the fluorescence of the NADH produced by the coupled enzyme reactions, increasing sensitivity. The observed fluorescence is proportional to the CK activity. However, one fluorescent band seen in some patterns does not represent CK activity. This nonspecific fluorescence, which co-migrates with albumin, is referred to as the "albumin artifact" or "family of artifacts" (5). Because the position of this band varies some with different sera, it can be mistaken for CK-BB activity. Several reports describe an apparent CK-BB in association with chronic renal disease, hemodialysis, and renal transplantation (6, 7).

Two methods have been proposed to eliminate the possibility of mistaking the band for CK-BB activity. One is to incubate the patient's serum with an antibody prepared against CK-BB before it is electrophoresed. A comparison of the patterns for treated and untreated serum shows a decrease in fluorescence for the sample treated with anti-CKBB if CK-BB is present (5). Another approach is to electrophorese the serum twice and examine the fluorescence with and without substrate (8). In this case, the inclusion of substrate results in an increase in fluorescence in the CK-BB region only for those sera having CK-BB activity. Both of these methods have demonstrated that the nonspecific fluorescence is not CK-BB.

A simpler method, which is better suited for routine use and more cost effective, enables one reliably to identify CK-BB without the need of a "blank" electrophoresis or the use of an expensive antibody reagent (9). The method involves use of a thin film of agarose gel (Corning AC1) for the electrophoretic separation of the isoenzyme pattern. The gel is produced by overlaying the gel with strips of chromatography paper (Whatman no. 542-SFC or Beckman PN no. 655295) saturated with CK substrate reagent. After a 30 min incubation at 41 °C, the overlay paper is removed from the gel, fixed in isopropanol, and air dried. The fluorescent pattern on the overlay paper is scanned for quantitation. Nonspecific fluorescence in the patient's serum contributing to the albumin-associated artifact and observed in the support media is not transferred onto the overlay paper, but remains in the gel.

Batch and column methods in which an ion-exchange resin is used to separate CK isoenzymes and measure them kinetically may be free of the artifact. However, these methods do not allow one to see the entire isoenzyme pattern. This advantage of the electrophoresis method is important in light of the well-documented reports of anomalous CK isoenzymes (10, 11).