Fig. 1. Method-comparison analysis by the Deming's method
Regression equations ("line of best fit"): a: \(y = 1.000x + 0.0, r = 0.997\), b: \(y = 0.997x + 3.25, r = 0.996\), c: \(y = 1.002x + 21.2, r = 0.997\), d: \(y = 0.741x + 0.169, r = 0.998\).

Fig. 2. Method-comparison analysis by the proposed method
Data from Figure 1 are replotted according to the proposed method.

Different interferences, which occasionally will give rise to erroneously high values for the analyte.

A constant error in one of the analytical methods to be compared (e.g., due to a constant contribution from a "blank") is indicated by an intercept value in the Deming plot, Figure 1c. A replot according to the proposed method (Figure 2c) clearly demonstrates this error. Figure 2c also shows that essentially the same values are obtained by the two compared analytical methods at values higher than 400 units.

The existence of a proportional error, as indicated by a slope deviating from unity in the Deming plot, Figure 1d, is also confirmed by the proposed plot for method-comparison analysis, Figure 2d.

I conclude that the proposed plot for method-comparison analysis is fast, and is a simple and reliable way of comparing the results.

References

Staffan Eksborg
Karolinska Pharmacy, Box 60024 S-104 01 Stockholm, Sweden

Chemical Distinctions among Body Fluids

To the Editor:

The origin of body fluids occasionally requires confirmation in the laboratory. Of particular concern are instances of rhinorrhea in which cerebrospinal fluid must be distinguished from nasal-lacrimal fluid, and cases when the sample needs to be determined to be peritoneal, serous, or amniotic fluids rather than urine from bladder fistulas or accidental puncture. Occasionally, urine must be distinguished from lymph or other fluids. Ordinarily, differentiation de-
pends on clinical symptoms, roentgenograms, pH measurements, and other factors that give less than satisfactory results. We have used the characteristics and distinctive chemical compositions of various body fluids to reflect significant differences and to provide a rapid and reliable method for distinguishing them.

We analyzed samples that had been submitted to the clinical chemistry laboratory for routine purposes over a four-week period. The specimens, kept frozen at −20 °C until use, were cerebrospinal fluid, urine, amniotic fluid, and serum. The serum samples included specially selected specimens to cover a wide, pathologically relevant range, from extremely high to extremely low. Lactrim fluid was unavailable for these studies. When indicated, specimens were centrifuged (850 × g, 3 min) to remove suspended matter from the body fluids.

We used a discrete automated analyzer, a reflectance spectrophotometer (Ektachem-4), and Ektachem reagent slides for total bilirubin (TBI), calcium (Ca), glucose (GLU), and urea nitrogen (BUN). Appropriate Ektachem standards were reconstituted to calibrate the Ektachem-4. Serum calibrators were used for serum samples; the other body fluids were assayed after both serum and aqueous calibrations. These standards, as well as known serum and urine controls, were analyzed in duplicate to serve as controls. Bilirubin, calcium, glucose, and urea nitrogen were assayed in duplicate on all examined fluids; sample volume was 25 μL.

The results of the determinations of these four analytes confirmed or extended those previously reported (1, 2). Excellent discrimination was obtained only in the case of urea nitrogen, and then only for urine vs the other examined fluids (Table 1). Although we obtained statistically significant differences between the means of values for the other tests, values overlapped when the entire range of values was considered, thus precluding using this basis for discrimination among these body fluids. Reliable distinction, however, was afforded by the urea nitrogen assay for urine from amniotic fluid: the differences between the means for urine and amniotic fluid were statistically highly significant (p < 0.0001). BUN values also discriminated between urine and serum (p < 0.01), with essentially no overlap over the entire ranges.

**Illustrative case report:** E.P., a 57-year-old white man, after having a left inguinal hernia repaired, developed infection and a gaping wound. Four days later the wound drainage increased markedly and was described as "yellow, watery fluid." The surgeon was concerned that this drainage might be urine from a bladder fistula, and he was preparing for extensive roentgenographic studies (intravenous pyelography and "sinus-gram"), cystoscopy, and, if necessary, laparotomy. The laboratory determined the concentration of urea nitrogen in the discharged wound fluid to be 180 mg/L; meanwhile, the patient's serum urea nitrogen concentration was 190 mg/L, as determined by a routine chemistry profile the preceding day. This result ruled out the possibility of the wound fluids being urine; consequently, the surgeon only debrided the wound, which subsequently healed over the next two weeks.

This approach, especially if extended to include additional chemical and protein variables, may allow rapid and precise discrimination of various body fluids of similar color and consistency.

### Table 1. Results (mg/L) for Four Analytes in Various Body Fluids

<table>
<thead>
<tr>
<th></th>
<th>Serum (n = 254)</th>
<th>Cerebrospinal fluid (n = 60)</th>
<th>Urine (n = 45)</th>
<th>Amniotic fluid (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBI</td>
<td>17</td>
<td>1–460</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Ca</td>
<td>87</td>
<td>45–160</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>GLU</td>
<td>1420</td>
<td>370–5050</td>
<td>660</td>
<td>290</td>
</tr>
<tr>
<td>BUN</td>
<td>270</td>
<td>30–1500</td>
<td>145</td>
<td>5550</td>
</tr>
</tbody>
</table>

In recent years, bandit et al. (1) described a clinical evaluation of a glucose/BUN analyzer. In their article, they state: "The 1976 FDA Proposed Class Standard Assay for Glucose was performed, with the following modification. Reagent kits for the hexokinase/glucose-6-phosphate dehydrogenase procedure were purchased from Calbiochem, La Jolla, CA 92037, and were used according to manufacturer's specifications."

The Calbiochem-Behring Kit is based on the same methodological principle as the Food and Drug Administration's (FDA's) proposed class standard (2), but the standard differs from the kit in six important particulars. The FDA proposed class standard requires (a) a Somogyi-protein-free supernate, (b) confirmation of the adequacy of ATP, NAD+, hexokinase, and glucose-6-phosphate dehydrogenase in the mixed reagent before it is used, (c) verification of the absence of interfering enzymes in the reagent, (d) macro sample and reagent volumes, (e) verification that instrumentation meets certain specifications, and (f) validation of the standard curve. Determination of glucose by the Calbiochem-Behring kit does not have any of these requirements. Furthermore, the final reaction concentrations of ATP, NAD+, and glucose-6-phosphate dehydrogenase in the Calbiochem-Behring Kit are only about half of the concentrations used in the FDA proposed class standard. In addition, Calbiochem-Behring does not claim that its glucose kit is the same as the FDA proposed class standard.

The misleading statement that the FDA proposed class standard was used in this evaluation is made repeatedly throughout the text (that is, in the abstract; materials, methods, and protocols section; Table 5; and Figure 2). An important use of validated and acknowledged candidate reference methods is in evaluating new methodological procedures. In this article, however, the Eastman glucose/BUN analyzer is compared with the Calbiochem-Behring Kit. The Eastman glucose/BUN analyzer is not compared with the candidate glucose reference method. Traceability of the Calbiochem-Behring Kit to the candidate glucose reference method is, however, demonstrated in Table 6.

The hexokinase method was one of three methods in the original FDA proposed class standard (3), and it was subsequently recognized as the reference method (4). The American Association for Clinical Chemistry and the National Committee for Clinical Laboratory Standards' National Reference System have also recognized this.

### References


**Medford T. McCoy, Melodie M. Finney, and Stephan E. Ritzmann**

*Clin. Chem. and Proteoinology*  
Dept. of Pathol.  
Baylor Univ. Med. Center  
Dallas, TX 75246

### Erroneous Reference to a Candidate Reference Method

**To the Editor:**

Recently, Bandi et al. (1) described a clinical evaluation of a glucose/BUN analyzer. In their article, they state: "The 1976 FDA Proposed Class Standard Assay for Glucose was performed, with the following modification. Reagent kits for the hexokinase/glucose-6-phosphate dehydrogenase procedure were purchased from Calbiochem, La Jolla, CA 92037, and were used according to manufacturer's specifications."

The Calbiochem-Behring Kit is based on the same methodological principle as the Food and Drug Administration's (FDA's) proposed class standard (2), but the standard differs from the kit in six important particulars. The FDA proposed class standard requires (a) a Somogyi-protein-free supernate, (b) confirmation of the adequacy of ATP, NAD+, hexokinase, and glucose-6-phosphate dehydrogenase in the mixed reagent before it is used, (c) verification of the absence of interfering enzymes in the reagent, (d) macro sample and reagent volumes, (e) verification that instrumentation meets certain specifications, and (f) validation of the standard curve. Determination of glucose by the Calbiochem-Behring kit does not have any of these requirements. Furthermore, the final reaction concentrations of ATP, NAD+, and glucose-6-phosphate dehydrogenase in the Calbiochem-Behring Kit are only about half of the concentrations used in the FDA proposed class standard. In addition, Calbiochem-Behring does not claim that its glucose kit is the same as the FDA proposed class standard.

The misleading statement that the FDA proposed class standard was used in this evaluation is made repeatedly throughout the text (that is, in the abstract; materials, methods, and protocols section; Table 5; and Figure 2). An important use of validated and acknowledged candidate reference methods is in evaluating new methodological procedures. In this article, however, the Eastman glucose/BUN analyzer is compared with the Calbiochem-Behring Kit. The Eastman glucose/BUN analyzer is not compared with the candidate glucose reference method. Traceability of the Calbiochem-Behring Kit to the candidate glucose reference method is, however, demonstrated in Table 6.

The hexokinase method was one of three methods in the original FDA proposed class standard (3), and it was subsequently recognized as the reference method (4). The American Association for Clinical Chemistry and the National Committee for Clinical Laboratory Standards’ National Reference System have also recognized this.