Table 3. Analytical Recovery for Three Concentrations

<table>
<thead>
<tr>
<th>Kit</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
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<td>126</td>
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</tr>
<tr>
<td>A</td>
<td>98</td>
<td>114</td>
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</tr>
<tr>
<td>SM</td>
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<td>154</td>
<td>116</td>
</tr>
<tr>
<td>CA</td>
<td>111</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>

Particular caution must be exercised when comparing results obtained with different reagent lots of the SM and CA kits.

References

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New Approaches to the Study of Proteinuria

To the Editor:

The need for new approaches for the study of proteinuria was recently described by Ellis and Buffone in your journal [Clin. Chem. 23, 666–670 (1977)]. Indeed, the amount of diagnostic information that can be obtained from adequate studies of proteinuria has generated considerable interest in the past few years. We do not think, however, that the answer lies exactly in the quantitative approaches outlined by Ellis and Buffone. Their approach, although it represents a step forward, still fails to take full advantage of the wide spectrum of indications that can be obtained from the study of proteinuria.

We have preferred qualitative approaches, and one of the most successful is sodium dodecyl sulfate/polyacrylamide gel electrophoresis. This technique, introduced by Waldmann’s group for the screening of tubular proteinuria (1) has proved to be one of the most efficient techniques for analytical characterization of proteinuria (2, 3). It is inexpensive, requiring only conventional disc electrophoresis equipment and a 100-μl aliquot of urine containing 150–200 μg of protein, a concentration often present in unconcentrated samples from patients with the nephrotic syndrome and one that can be easily achieved by any standard concentration procedure when the protein concentration is insufficient.

The great potential of this technique is illustrated in Figure 1, which shows the separation of proteins in several urines from diabetic patients (in part of a screening program for diabetic nephropathy being done in the Division of Endocrinology). The separations obtained in gels 1–3 are within normal limits, consistent with a normal 24-h proteinuria. The separations on gels 4 to 7 illustrate various degrees of glomerular proteinuria; one of the urines shows relatively large amounts of IgG (gels 6 and 7), suggesting poor selectivity.

The fact that some of these patients still show a quantitatively normal proteinuria does not invalidate the conclusion of glomerular damage based on the electrophoretic separation. The urine separated on gel 18 shows mixed proteinuria, with both components of glomerular origin and low-molecular-weight proteins, suggesting tubular damage. Finally, the urine separated on gel 9 shows low-molecular-weight proteinuria, later identified by immunochemical methods as Bence Jones protein of kappa type. This is the second case of asymptomatic Bence Jones proteinuria detected in our screening of abnormal proteinuria in diabetic patients, of 52 patients screened. If we consider that all these patients, when screened by routine methods, were judged to have only trace proteinuria, the advantage of a sensitive and informative method such as this becomes evident.

Quantitative data are of unquestionable value. Total 24-h proteinuria is still one of the best indicators of renal damage, and determinations of specific proteins, as proposed by Ellis and Buffone, will always be of interest. On the other hand, selection of individual proteins for quantitative assay should not be based on convenience or ease of assay, but rather on the potential usefulness of the data to be obtained. In our experience, it is of advantage to assay at least one high-molecular-weight and one low-molecular-weight protein. Among the latter, lysozyme is easy to assay by either the lysozyme method (4) or the immunonephelometric method recently reported by one of us [Clin. Chem. 23, 967 (1977)], and the increase of this enzyme in tubular damage has been well documented (5). However, the correlation between lysozymuria and tubular proteinuria as detected by sodium dodecyl sulfate/polyacrylamide gel electrophoresis is not always perfect (6), and so the two methods are actually complementary. For high molecular weight, instead of IgG or α1-macroglobulin we prefer to assay high-density lipoproteins, which can be found in urines of patients with severe glomerular damage, the amount excreted correlating well with the selectivity index (Figure 2), as calculated from the clearances of IgG and transferrin (7). High-density lipoprotein determination has advantages over the classical calculation of selectivity indexes, because (a) it involves a single determination of one protein in the urine, (b) it does not seem to be af-

Fig. 1. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis study of the urinary proteins of eight diabetic patients

The first two gels on the left were used to separate reference proteins. In gel C we separated several purified proteins of known molecular weights: IgM (1,000,000), IgG (153,000), transferrin (78,000), serum albumin (66,000), egg albumin (OVA, 48,000), chymotrypsinogen A (CHY, 28,000), and lysozyme (LZM, 15,000). Normal human serum was separated in the next gel (S). The next nine gels were used to separate the urinary proteins of eight different patients. One of them was studied in duplicate in gels 6 and 7. The total proteinuria of each patient is indicated on the bottom of each gel.
References


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Adaptation of EMIT Phenyltoin Assay to the Perkin-Elmer 124 D Spectrophotometer

To the Editor:
We would like to report this procedure for others who have this instrument available and would like to use the EMIT (Syva) system.

Hardware: (1.) Perkin-Elmer (P.E.) double-beam spectrophotometer Coleman Model 124 D. (2.) Enzyme Calculator, Coleman 5-100. (3.) BCD Printer Model Coleman 5-300. (4.) Autofill-cell assembly system Coleman Model 124-81000 (this assembly includes: (a) sampling head assembly (sipper) No. 124-81100; (b) microcell holder No. 124-84000; (c) systems pump, Coleman Model 5-300; (d) microflow cell jacketed 10 mm-glass P.E. No. 005-0103]. (5.) Automatic pipette, Micromedic Systems No. 250006. (6.) Constant temperature bath Lauda k-2/RD (Brinkmann Instruments).

Reagents: (1.) EMIT; phenyltoin (6 B019; Syva Corp., Palo Alto, Calif. 94303). (2.) EMIT; AED Calibrators (Syva Corp.).

Procedure: (1.) Set spectrophotometer at 340 nm with the deuterium lamp as a light source. (2.) Set enzyme calculator for 8 x 15 s intervals with kF at 25 and kP multiplier at 100. (3.) Adjust the systems pump to aspirate approximately 1.4 ml into the flow cell via the sipper. (4.) Set the automatic pipette to aspirate exactly 50 ml and to deliver this plus 500 ml of buffer. (5.) Adjust the constant temperature bath to 30.0 ± 0.1°C.

The procedure outlined in the kit insert then is followed exactly, except that all dilutions are made by adding 50 ml to 500 ml of buffer instead of the recommended 250 ml. Thus, 50 ml of sample is added to 500 ml of buffer and 50 ml of this is again added to 500 ml of buffer. Fifty microliters of solutions A and B are diluted with 500 ml of buffer by using the Micromedic dilutor. After the last step (addition of 50 ml of solution B—enzyme/drug complex—to the mixture of solution A and diluted specimen) the sample is aspirated immediately into the jacketed flow cell, which activates the enzyme calculator and printer. The difference between the absorbance after 15 s and after 120 s (AA) is calculated.

The results thus obtained with the calibrators, plotted on the logit-log plot provided, resulted in a linear calibration curve. Values for individual samples were calculated from this curve. Sixty-six random samples were determined by the above-described procedure and by a spectrophotometric method. The values were compared by least-squares regression, yielding a line with a slope of 1.05, intercept of 0.64 mg/liter, standard error of 2.74 mg/liter, and a correlation coefficient of 0.98.

References


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Simple Pattern-Recognition Format for Laboratory Tests

To the Editor:
The central role of a clinical laboratory is to provide objective evidence to the physician so that sound judgments can be made. A single biochemical test is, with rare exceptions, of little diagnostic value to the physician. This is especially true of serum enzyme assays. The recent development of high-speed analyzers that require only microsamples and small volumes of reagents has drastically decreased the reagent cost. By changing to one of these systems