Interlaboratory Comparison of the Measurement of Albumin in Urine
Patricia W. Mueller, Mary Louise MacNeill, S. Jay Smith, and Dayton T. Miller

Because of increased interest in the assay of albumin in urine and the sensitivity required to quantify concentrations associated with (a) increased risk of developing end-stage renal disease and cardiovascular disease among people with diabetes and (b) renal damage caused by exposure to nephrotoxic substances, we conducted a pilot study of the variation of these measurements within and among five laboratories that use various immunoassays. These assays included two different enzyme immunoassays, two different immunoturbidimetric assays, a fluorescent immunoassay, and a zone immunoelectrophoresis assay. The results indicate considerable variation both within and among laboratories for measurements at or near the normal range. Variability is equally attributable to the precision of individual immunoassays and to the variation of the mean values obtained by each laboratory. Individual laboratory CVs ranged from 5.8% to 18.2% for mid- and high-concentration samples treated with preservative and from 8.4% to 23.6% for mid- and high-concentration samples containing no preservative. The relative bias of individual laboratory means ranged from −56.4% to 20.5% for the two preserved materials and from −32.6% to 0.8% for the two materials containing no preservative. To reduce the chance of misdiagnosing the risk associated with above-normal albumin concentrations in urine, we need to address the problems contributing to imprecision and inaccuracy, particularly laboratory-to-laboratory variability.

Additional Keyphrases: variation, source of • assessing renal function • enzyme immunoassay • fluoroimmunoassay • immunoelectrophoresis • zone immunoelectrophoresis

Clinicians and researchers are increasingly interested in the analytical determination of the protein albumin in urine because (a) increased values indicate an increased risk of developing end-stage renal disease (1-3) and cardiovascular disease among people with diabetes (1-4) and (b) albumin in urine is a sensitive indicator of renal damage caused by exposure to nephrotoxic substances (5-14). A recent consensus statement by representatives of the Centers for Disease Control and the National Institute of Diabetes and Digestive and Kidney Diseases on the kidney disease aspect of diabetes mellitus included the recommendation that albumin in the urine of people with diabetes be routinely monitored (15). Because variations in the measurement of albumin concentrations in urine may result in misclassification of a person’s risk of developing life-threatening complications, we began a pilot study to examine the extent of these variations.

In healthy subjects, albumin is ordinarily present in human urine in concentrations in the low mg/L range, with sustained values greater than about 15 to 30 mg/L usually being regarded as abnormal (1, 16-23). Methods used for routine measurement involve several types of immunoassay procedures, with various methods being used in different clinical and research settings. High biological variability in amounts of albumin excreted (usually calculated per unit of time or as a ratio to creatinine excretion) has been reported (15, 20, 24-27). The role that assay variability plays in these observations, however, is not clear.

We, therefore, conducted a pilot interlaboratory comparison of the measurement of albumin in urine to estimate the degree of variability among and within laboratories. Participating were five laboratories performing albumin measurements for studies of diabetes, hypertension, and environmental and occupational health, and for the third National Health and Nutrition Examination Survey (NHANES III). These laboratories used a variety of methods, including two different enzyme immunoassays, two different immunoturbidimetric assays, a fluorescent immunoassay, and a zone immunoelectrophoresis assay. The study was designed to estimate existing variability, not to compare methods. We prepared albumin reference materials (28), taking into consideration the following important factors for the production of materials containing high-molecular-mass compounds for which no reference or definitive method exists: (a) use of the same type of matrix and protein as in the specimens to be assayed; (b) determination of the allowable variability of the target value of the materials by consensus, if necessary; and (c) examination of the need for special conditions, such as special matrix requirements, treatment of matrix, and stability under storage conditions (29).

Materials and Methods

Preparation of materials. We prepared materials to contain three concentrations of albumin in the normal range (target values: 2.38, 15.38, and 29.38 mg/L), using Tween 20 (polyoxyethylene sorbitan monolaurate, cat. no. P 1379, Sigma Chemical Co., St. Louis, MO), as a preservative (28). We also prepared materials with no preservative at three comparable concentrations of albumin (target values: 1.10, 14.10, and 26.10 mg/L) for use when we suspected interference by the preservative.
We screened urine samples from 21 donors for low albumin content and for matrix stability, and chose donors from this group. We then collected and sterile-filtered urine from these donors and supplemented it with human serum albumin (Cohn Fraction V; Miles Scientific, Naperville, IL). Tween 20 was also added to the preserved materials. We aliquoted both materials and stored the preserved materials at −20°C and the unpreserved materials at 4°C. Both sets of materials were stable for the duration of the study, i.e., showed no statistically significant loss of albumin by enzyme immunoassay.

Purity of supplemented albumin. We verified the purity of the supplemented human serum albumin by a modification (30) of the protein assay of Lowry et al. (31), using standards of bovine serum albumin (National Institute of Standards and Technology, Gaithersburg, MD; Standard Reference Material 926) (28). The mean difference between the protein found and the protein expected in assays of five albumin concentrations ranging from 3.1 to 50.0 mg/L was −5%, which was within the observed CV of the Lowry assay (7.9%). In addition, the supplemented material exhibited only one band by agarose gel electrophoresis (28).

Determination of the target values. The target values of the low-concentration materials are the means of our enzyme immunoassay results (28). The target values of the mid- and high-concentration materials are the sums of the endogenous albumin content and the amount of supplemented albumin.

Design of the study. We distributed 20 vials from each of three concentrations of the preserved materials to the participating laboratories. The preserved materials were shipped on solid CO2, and the unpreserved materials were shipped on ice packs. Accompanying instructions stated that each laboratory should assay no more than two aliquots of a given concentration in one day. The reports we received included individual replicates of each determination in addition to the mean value, except for laboratory C's report on the preserved materials, which included only the means of duplicates. Participants phoned in the test results for the first three vials of each concentration so that we could check for major problems and potential interference of the preservative.

Albumin assays. Laboratory A used the immunoturbidimetric assay of McCormick et al. (32); laboratory B, an enzyme-linked immunosorbent assay for albumin that was a modification (28) of an immunoassay by Fielding et al. (33) and Whitfield and Spierto (34); and laboratory C, the Albuwell enzyme immunoassay kit (Exocell, Philadelphia, PA 19104). Laboratory C also used the SPQ Test System for Microalbumin, an immunoturbidimetric assay kit (Atlantic Antibodies, Scarborough, ME). Laboratory D used the fluorescent immunoassay of Chavers et al. (35), and laboratory E used the zone immunoelectrophoresis assay of Vesterberg (16).

Statistical analysis of results. We entered the results from participants into a computer file and verified them by comparison with the original reports. We used analysis of variance to apportion the total observed variation in measured concentration into among-laboratory and within-laboratory sources. Because no two laboratories used identical methods, the observed variabilities are not necessarily characteristic of a given method. The components of variance are expressed as percentages of total variance from all sources and as CV values. The among-laboratory and within-laboratory variance components sum to 100%. We calculated the relative bias by subtracting the target value from the observed value and dividing the remainder by the target value.

Results
The results of this study indicate considerable variation both within and among laboratories (Figure 1). We found similar patterns of precision and bias at each
concentration of preserved material for the various laboratories. The means from three laboratories were consistently above, and the means from two laboratories were consistently below, the calculated target values. The only significant change in these patterns was the greater variability of the immunoturbidimetric method reported by laboratory A for the low-concentration material (Figure 1, bottom). Although laboratory A chose to report these values, the variability is probably greater because of higher detection limits characteristic of immunoturbidimetric methods. When the laboratories reported their initial three values by phone, we detected the low values of laboratory C. We then sent this laboratory unfrozen materials with no preservative, to evaluate the possibility that the preservative was causing the low values. The laboratory assayed the unpreserved materials by an enzyme immunoassay and an immunoturbidimetric assay. These results are shown in

![Graphs showing bias from target value for different laboratories.](image)

Table 1. Total Among- and Within-Laboratory Variation*

<table>
<thead>
<tr>
<th>Target value, mg/L</th>
<th>Among-laboratory</th>
<th>Within-laboratory</th>
<th>Total</th>
<th>Among-laboratory</th>
<th>Within-laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variance, %</td>
<td></td>
<td></td>
<td>CV, %</td>
<td></td>
</tr>
<tr>
<td>2.38</td>
<td>43.8</td>
<td>56.2</td>
<td>32.9</td>
<td>21.8</td>
<td>24.7</td>
</tr>
<tr>
<td>15.38</td>
<td>47.3</td>
<td>52.7</td>
<td>17.9</td>
<td>12.3</td>
<td>13.0</td>
</tr>
<tr>
<td>29.38</td>
<td>51.4</td>
<td>48.6</td>
<td>18.8</td>
<td>13.5</td>
<td>13.1</td>
</tr>
</tbody>
</table>

* For the preserved materials assayed by five different laboratories.

comparison with values obtained by a different enzyme immunoassay (Figure 2). The means obtained by laboratory C for the mid- and high-concentration materials with no preservative are also less than the calculated target values, whereas the means for the low-concentration material by enzyme immunoassay are comparable with the target values.

A statistical analysis of variance showed an approximately equal distribution of variance within and among laboratories. For the preserved materials the total variance due to among-laboratory variation was 43.8% to 51.4% and that due to within-laboratory variance was 46.6% to 56.2% (Table 1). The total among-laboratory CV was greatest for the lowest albumin concentration and was about equal for the higher albumin concentrations. The total within-laboratory CV followed a similar pattern. The total CVs for the mid- and high-concentration materials were also about equal (Table 1).

The individual within-laboratory CVs ranged from 9.0% to 44.8% for the low-concentration preserved material, from 5.7% to 18.0% for the mid-concentration material, and from 5.1% to 15.8% for the high-concentration material (Table 2).

The relative bias of the individual laboratory means ranged from −51.9% to 19.1% for the mid-concentration preserved material (means: 7.4 to 18.3 mg/L) and from −56.4% to 20.5% for the high-concentration material (means: 12.8 to 35.4 mg/L) (Table 3).

![Graph showing percent difference from mean.](image)

**Table 2. Individual Within-Laboratory CVs**

<table>
<thead>
<tr>
<th>Laboratory (method)</th>
<th>Low-conc</th>
<th>Mid-conc</th>
<th>High-conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (TUR)</td>
<td>44.8 (38)</td>
<td>18.0 (38)</td>
<td>15.8 (36)</td>
</tr>
<tr>
<td>B (EIA)</td>
<td>13.0 (198)</td>
<td>13.0 (198)</td>
<td>13.8 (198)</td>
</tr>
<tr>
<td>C (EIA)*</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D (FIA)</td>
<td>9.0 (38)</td>
<td>7.8 (34)</td>
<td>7.2 (40)</td>
</tr>
<tr>
<td>E (ZIA)</td>
<td>12.4 (40)</td>
<td>5.7 (40)</td>
<td>5.1 (40)</td>
</tr>
<tr>
<td>Unpreserved</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (EIA)</td>
<td>14.9 (50)</td>
<td>15.2 (52)</td>
<td>16.8 (50)</td>
</tr>
<tr>
<td>C (EIA)</td>
<td>38.4 (20)</td>
<td>22.9 (16)</td>
<td>12.2 (20)</td>
</tr>
<tr>
<td>C (TUR)</td>
<td>NR</td>
<td>21.7 (20)</td>
<td>8.2 (20)</td>
</tr>
</tbody>
</table>

* The differences in within-laboratory variability are statistically significant (P < 0.01) except for the mid-concentration unpreserved material.

** Table 3.** 193
When the three materials without surfactant were evaluated by the laboratories B and C, which used two different enzyme immunoassays and an immunoturbidimetric assay, the laboratory CVs ranged from 14.9% to 38.4% for the low-concentration material, from 15.2% to 22.9% for the mid-concentration material, and from 8.2% to 16.6% for the high-concentration material (Table 2). The range of relative bias was −32.6% to −0.7% for the mid-concentration material and −25.7% to 0.8% for the high-concentration material (Table 3).

**Discussion**

Imunoassays are inherently less precise and more subject to bias than other chemical and physical techniques in the clinical laboratory; however, the information that they yield can be of great clinical value (36, 37). The increasing use of these tests for the diagnosis and treatment of disease dictates that we give particular attention to improving precision and accuracy to the extent possible. This study indicates the potential for considerable laboratory-to-laboratory variation in the measurement of albumin concentrations in urine in the normal or near normal range. Because many researchers consider values above 15 to 30 mg/L to be clinically significant (1, 16–23), variations of the magnitude seen here would result in a large potential for misclassification of risk. The albumin materials used in this study were prepared to minimize variability by careful matrix selection and preparation (28). We have found in a study of 21 apparently healthy people that the variability of individual samples was considerably greater than the variability observed with materials prepared as these were (28).

The sources of the high observed variability are two-fold: the precision of the individual immunoassays and the variation of the mean values obtained by each laboratory. Precision and accuracy contribute equally to the error observed in this study. Factors affecting the precision and accuracy of immunoassays include instrument or operator failure; lack of consistency in supplies, standards, and other reagents used; the portions of the standard curves used; methodology; sample storage and preparation; and dilution of samples (36, 37). Many of these factors undoubtedly contribute to the variability seen here. Although determination of which factors make the greatest contribution requires detailed evaluation of laboratories and methods, three specific needs are evident: (a) a clear definition of the lower detection limits of various assay methods, (b) an improvement in laboratory-to-laboratory variability, and (c) an improvement in precision. Recommendations for analytical CV goals, based on estimates of one-half the intra-individual biological variation, range from 12% (38) to 18% (20, 39).

We are now characterizing new materials prepared similarly to the unprepared materials prepared in this study for use in the continuing assessment of these laboratories. The preserved materials used in this study as well as the new unprepared materials are also being characterized by another commonly used method, radioimmunoassay.

**References**


