I describe a rapid, sensitive immunoturbidimetric assay for measuring urinary albumin and immunoglobulin G with use of an automated spectrophotometer. Diluted urine samples and polyethylene glycol in phosphate-buffered saline are pipetted into the cuvettes of the spectrophotometer. The initial absorbances of the samples are measured at 340 nm; antiserum to albumin or to immunoglobulin G is added to each tube, and after 2 min at 37 °C the absorbance of the mixtures is read at 340 nm. The initial blank absorbances of the samples are subtracted from the final absorbances automatically. The change in absorbance is linear with concentration in the range of 5–400 mg/L for albumin and 3–1000 mg/L for IgG. The lower limit of the determination is 5 mg/L for albumin, 3 mg/L for IgG. Linear correlations were observed between the concentrations of albumin and immunoglobulin G determined by this method (x) and those determined by radial immunodiffusion (y). The regression equation for albumin was y = 0.84x + 0.03 (r = 0.99, n = 87), and for IgG y = 0.94x + 0.02 (r = 0.98, n = 87).

Determination of urinary total protein excretion is not sufficient for accurate diagnosis of renal disease. To monitor kidney function and evaluate the degree of glomerular dysfunction, clearance studies of individual proteins of various molecular masses are needed (1–3). The selectivity of protein excretion is estimated by plotting the clearance of the various proteins vs their molecular masses on a logarithmic scale. The slope of this regression line is used as a measure of selectivity. Selectivity provides information on the state of the glomerular filter, which is useful in clinical practice for the assessment of glomerular damage, for the management of the nephrotic syndrome in childhood, for differentiating between chronic pyelonephritis and chronic glomerulonephritis and between proliferative and minimal-change glomerulonephritis. Ideally, clearances of several proteins, representing a wide range of molecular sizes, are used to measure the selectivity. However, for most clinical purposes selectivity can be estimated accurately enough on the basis of the clearances of albumin and IgG (4).

Radial immunodiffusion (5) and electroimmunoassay (6) have been used most frequently for quantitatively determining various urinary proteins. Electrophoresis on polyacrylamide gel in the presence of detergent (7) gives a rough estimate of the ratios of proteins of different molecular sizes. Radioimmunoassay has been used for the quantitative determination of albumin, IgG, and β2-microglobulin in urine (2, 8, 9), and nephelometry for the determination of various plasma proteins in urine (10, 11). All these methods are fairly sensitive but rather cumbersome, time consuming, and expensive, and except for nephelometry, they are difficult to automate.

This paper presents an easy and rapid method for the quantitatively determining urinary proteins, based on the formation of insoluble antigen–antibody complexes in the presence of excess antibodies; complex formation is accelerated with polyethylene glycol. The absorbances of the initial urine samples is subtracted automatically, and the microturbidity caused by the immunocomplexes is measured. This change in absorbance varies linearly with the amount of antigen in the urine sample. Measurements of the concentrations of albumin and IgG in 87 urine samples by this method are compared with those obtained by radial immunodiffusion.

Materials and Methods

Reagents. Polyethylene glycol 6000 (Carbowax 6000) was obtained from Fluka AG, Buchs SG, Switzerland. Tween 20 (polyoxyl persecorbisorbita monolaurate) and trichloroacetic acid were from Sigma Chemical Co., St. Louis, MO 63178. The antisera to human albumin and IgG were from Orion Diagnostica, Espoo, Finland.

Urine samples. Urine was collected from 87 patients with renal dysfunctions (mean age, 47.9 years). Specimens were centrifuged and stored at 4 °C, with thymol crystals added as a preservative, for one to two days before analyses. The samples were neither filtered nor concentrated. Seven samples had total protein concentrations of less than 0.1 g/L; the other 80 had concentrations ranging from 0.1 to 9.7 g/L (mean, 1.6 g/L).

Assay buffer. This was phosphate-buffered saline, pH 7.3, containing, per liter, 50 mmol of phosphate, 150 mmol of NaCl, 40 g of polyethylene glycol 6000, 0.2 mL of Tween 20 surfactant, and 1 g of NaNS.

Antisera. Highly avid antibodies to human albumin and to IgG heavy chains were isolated from swine antisera by precipitation with ammonium sulfate and by the use of immunosorbents: albumin or γ-heavy chains coupled to cyanogen bromide-activated Sepharose (Pharmacia, Uppsala, Sweden). The immunoglobulins were dissolved in phosphate-buffered saline and used as antisera. Their titers, as measured by radial immunodiffusion (12), were 2 g/L for antibodies to albumin and 3 g/L for antibodies to IgG.

Standards. Standard preparations were diluted with physiological saline from human protein reference serum (Orion Diagnostica).

Other methods. Radial immunodiffusion (5) was used as a reference method for the determination of albumin and IgG. The total protein concentration of urine was measured by the biuret method (7) after precipitation of proteins with trichloroacetic acid (100 g/L).

Apparatus. The spectrophotometer used was a System Olli 3000, photometer 334, equipped with thermostated incubators, mixer devices, and an automated sample- and reagent-dispensing device, System Olli 3000, dispenser 216 (all from Ollitute Oy, Espoo, Finland).

Albumin determination. Pipet 50 μL of undiluted standards and of urine diluted 10-fold in physiological saline into cuvettes of the Olli analyzer, and add 850 μL of assay buffer to each tube. After incubation for 10 min at 37 °C, measure the initial absorbances at 340 nm. Then add 100 μL of undiluted antiserum to albumin to each tube with the automated dispenser. After 2 min read the absorbances at 340 nm and subtract the initial absorbances.
**IgG determination.** IgG determinations are performed in the same way, except that the urine samples and antiserum to human IgG are undiluted.

**Results**

**Incubation interval.** The effect of the duration of incubation on the quantity of antigen–antibody complexes precipitated at different concentrations of albumin and IgG was studied, with use of undiluted antiserum. The turbidity at 340 nm increased rapidly during the first 2 min, but then remained constant for at least for the next 6 min for albumin concentrations up to 400 mg/L and for IgG concentrations up to 1000 mg/L (Figure 1). A 2-min incubation was used in all further experiments.

**Linearity.** Typical standard curves for albumin and IgG are presented in Figure 2. With undiluted antiserum the change in absorbance increased steadily (but not linearly) with albumin concentration over the range 0.05–0.4 g/L. The method can thus be used to measure the concentration of albumin over this range for undiluted urine and over the range 0.5–4.0 g/L for 10-fold diluted urine. With undiluted antiserum the concentration of IgG over the range 0.01–1.0 g/L can be measured. With antiserum diluted twofold the upper limit of concentrations to be measured are 0.2 g/L and 0.5 g/L for albumin and IgG, respectively.

**Sensitivity.** The lower limit of the determination is 50 mg of albumin per liter at a 10-fold sample dilution, and 5 mg of albumin and 3 mg of IgG per liter with undiluted urine.

**Precision.** Two urine samples with albumin concentrations of 3500 and 90 mg/L, respectively, were assayed in 11 separate assays, to assess between-run precision. The coefficients of variation (CVs) were 5.7 and 6.9%, respectively. The corresponding within-assay CVs obtained in 24 parallel determinations were 5.6 and 7.5%. Repeated assay of two urine samples with IgG concentrations of 500 and 30 mg/L yielded within-assay CVs of 5.4% for both samples (n = 24), and between-assay CVs of 9.9 and 11.1% (n = 11), respectively.

**Recovery studies.** Various amounts of human serum with
known concentrations of albumin and IgG were added to protein-free urine and assayed. The results are presented in Table 1.

**Comparison with immunodiffusion.** In 87 urine samples with albumin values of 20 to 8700 mg/L, the results obtained by immunodiffusion (y) and by the method presented here, (x) showed a linear correlation \( r = 0.99 \), the regression equation being \( y = 0.84x + 0.03 \). For urinary IgG concentrations ranging from 3 to 1100 mg/L, the regression equation was \( y = 0.94x + 0.02 \) \( r = 0.98 \).

**Discussion**

This immunoturbidimetric assay, previously used to measure the concentrations of proteins in sera \((13,14)\), provides an easy and rapid method for measuring many urinary proteins.

The mean amount of albumin excreted in 24 h by normal individuals is around 6 mg \((10)\), and the amount of IgG 0.6–9.1 mg \((2)\). The range of albumin concentration covered in this assay is 5–4000 mg/L and that of IgG 3–1000 mg/L. Therefore the assay allows quantitative determination of urinary albumin and IgG even in individuals with normal renal function \((9,13)\). The assay can be used to simultaneously determine proteins in serum and urine, and is thus an elegant method for obtaining data for use in assessing the selectivity of proteinuria and for estimating the degree of glomerular dysfunction.

The method is easy and rapid. With a good spectrophotometer equipped with an automated sample- and reagent-dispensing device, more than 200 determinations can be performed in 20 min. Because the turbidity remains constant for at least 6 min after the precipitation in complete, the assay can also be used in laboratories having less automation.

The assay is much less costly than radioimmunoassay, and results are comparable with those by radial immunodiffusion. In this paper I used a rather high concentration of antisera, to increase the linearity of the assay and to avoid time-consuming dilution of the samples, but costs can be further reduced by using diluted antiserum and samples.

**References**


### Table 1. Analytical Recovery of Albumin and IgG Added to Protein-Free Urine

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<tr>
<th>Added</th>
<th>Measured</th>
<th>Recovery, %</th>
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