CLIN. CHEM. 36/3, 446–449 (1990)

Rapid, Robust Method for Measuring Low Concentrations of Albumin in Urine

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We describe a rapid particle-enhanced turbidimetric immunoassay for albumin in urine. Intra- and interassay CVs were <5% and <10%, respectively, the detection limit is 2 mg/L, and the working range extends to 200 mg/L. Mean analytical recovery of albumin added to centrifuged urines was 100% (SD 10.6%), and, when results were compared with those by the Pharmacia RIA, the correlation coefficient was 0.99. The working reagents are stable for at least six months; thus this assay is suited for both batch and urgent analysis.

Additional Keyphrases: *particle-enhanced turbidimetric immunoassay · "microalbuminuria" · calibration stability · diabetes*

Measurement of proteins and enzymes in urine is becoming increasingly important in the detection of renal pathology (*1*). The appearance of increased concentrations of certain proteins in urine has been used to assess glomerular permeability and tubular damage (*2*). Moreover, slightly increased excretion of albumin in urine ("pauci-albuminuria") has been recognized as an early indicator of renal damage in diabetes (*3*)—a situation that can be reversed if detected and treated sufficiently early (*4*).

Heretofore, methods for measuring low concentrations of albumin have been confined to heterogeneous immunoassays involving various labels such as radioisotopes (*5*), enzymes (*6*), and fluorophores (*7*). More recently, homogeneous immunoturbidimetric methods have been described (*8, 9*). Here we describe the development and validation of a rapid latex particle-enhanced immunoturbidimetric assay (PETIA), that can be adapted for use with most automated instruments in the clinical laboratory.

Materials and Methods

Materials

The latex particles were from E.I. du Pont de Nemours, Wilmington, DE. Human albumin was from Behring, Somerville, NJ. Antiserum, raised in goats against human albumin, was obtained as the IgG fraction from Atlantic Antibodies, Scarborough, ME 04074.

The latex particle–antibody conjugate was produced with 40-nm (diameter) latex particles, based on the polyvinyl naphthalene core described by Litchfield et al. (*10*). The chemically reactive shell was prepared with a mixture of 2-vinylnaphthalene and chloromethylstyrene (90/10 by vol). The IgG fraction of the antiserum was mixed with the latex particle suspension to yield a final immunoglobulin concentration of 4 g/L in phosphate buffer (15 mmol/L, pH 7.5) containing, per liter, 0.5 mL of GAFAC RE610, surfactant (GAF Corp., Wythenshaw, Manchester, U.K.) and 5 g of particles. The mixture was incubated at 37 °C overnight, and free IgG was then removed by washing the antibody-coated particles four times in 50 mmol/L glycine solution (pH 7.5), then centrifuging at 40 000 × *g* for 50 min to sediment the particles. The antibody–particle complex was finally resuspended in half the original volume of glycine solution (200 mmol/L, pH 7.5) for storage. Before use, the particles were sonicated twice for 1 min at full power in a "Soniprep" (MSE Instruments, Sussex, U.K.).

Instrumentation. All reactions were monitored with a Multistat III microcentrifugal analyzer (Instrumentation Laboratory, Lexington, MA) (*11*) at 340 nm and 30 °C, in a 0.5-cm (pathlength) cuvette.

Procedures

The assay protocol for all the experiments is similar to the final protocol described below, with appropriate variation of the conditions.

Final assay protocol. Into the inner compartment of the Multistat rotor dispense 5 μL of urine plus 89 μL of phosphate buffer (340 mmol/L, pH 7.5) and 4 μL of water. Into the outer compartment dispense 200 μL of particle reagent, diluted to give an initial absorbance of 0.7 in the reaction cuvette, and 10 μL of water. Incubate at 30 °C, then centrifuge to mix the reactants. Measure the absorbance at 340 nm immediately (3 s) and 240 s after this mixing.

Effect of buffer type, concentration, and pH. The effect of pH on the rate of aggregation was assessed by using various buffers in the pH range 6.0 to 10.0. The reaction was monitored by using antibody-coupled particles in the presence and absence of urine sample. The maximum aggregation in the presence of urine was noted at pH 7.4, none was noted in the absence of urine. Nonspecific aggregation did not occur at any point within the pH range studied.

Effect of polyethylene glycol. We investigated the effect of PEG 8000 (Union Carbide Corp., Danbury, CT) on the rate of reaction, using concentrations ranging from 0 to 40 g/L in the final reaction mixture. The 10 g/L concentration finally chosen was a compromise between the turbidity produced with added sample and the speed of the reaction.

Antibody loading on particle. Antibody was coupled to particles in a series of experiments covering a protein:particle ratio ranging from 0.5 to 4.0 g of antibody per 5 g of particle per liter of reagent. Each antibody–particle reagent was then used to establish a calibration curve in the assay. The detection limit was lowest and the equivalence point highest with 4 g of antibody per liter loaded onto the particle reagent.

Results

Precision. We assessed within-run precision by analyzing 19 aliquots of each of three different urine specimens. We assessed between-day precision by analyzing one aliquot of each of the three pooled specimens of urine on 20 consecutive working days. Aliquots of the urine were stored at 4 °C until use. We calculated the CVs by using a calibration curve prepared on the first day of the month and also by

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Received August 16, 1989; accepted December 11, 1989.

using a curve prepared on each of the 20 days (Table 1).

Calibration range, stability, and detection limit. Isotonic saline containing 1000 g of human albumin per liter was serially diluted with saline and assayed. Figure 1 indicates an equivalence point of approximately 200 mg/L, but shows that a concentration of 300 mg/L still gives an absorbance change exceeding that given by the 100 mg/L standard.

A series of albumin calibrators covering the range 5 to 200 mg/L were analyzed over six months. Figure 2 shows the absorbance changes during this time. The calibrators were prepared in isotonic saline containing 10 μL of Brij 35 surfactant (Sigma Chemical Co., St. Louis, MO) per liter, and then stored at 4 °C in small plastic tubes until use.

The detection limit, defined as 3 × the SD for the reagent blank signal, was 2 mg/L, but could be improved to <0.5 mg/L by increasing the sample volume to 50 μL.

Method comparison. Urine specimens collected from a series of patients were assayed for albumin by the proposed and by the Pharmacia radioimmunoassay methods (Pharmacia Diagnostics, Uppsala, Sweden). The results are shown in Figure 3.

Recovery of albumin. A stock of pure human albumin, prepared in isotonic saline containing 10 μL of Brij per liter, was used to supplement 10 urine samples with three concentrations of albumin. On assay the mean analytical recovery was 100% (SD 10.6%) when the samples were centrifuged before analysis and 96% (SD 33.4%) when they were not.

Comparison with nonenhanced turbidimetric immunoassay. Using the same antiserum, we developed a direct immunoturbidimetric assay based on conventional criteria (*12*). A 20-μL sample was needed to give an acceptable signal when the sample was mixed with 125 μL of antiserum diluted 50-fold in phosphate buffer (100 mmol/L, pH 7.4) containing 40 g of PEG 6000 per liter. The calibration curve is compared with that of the particle-enhanced assay in Figure 4, and demonstrates the improvement in signal and larger assay range that the latex-enhanced assay provides.

Discussion

The use of particles as labels in the design of homogeneous immunoassays is well described. Various particles have been used for this purpose, including erythrocytes (*13*), bacteria (*14*), gold sols (*15*), and latex (*16*). Despite the large amount of literature on this subject that has appeared during several decades, only relatively recently have light-scattering immunoassays been introduced for the quantitative assay of constituents in serum and urine.

Many latex-enhanced immunoassays have been described for both large and small molecules, involving use of particles of varying size, core material, and shell chemistry. Litchfield et al. (*10*) described a 40-nm-diameter particle with a polyvinylnaphthalene core and chloromethyl-

Table 1. Precision Data for the Present Method for Urinary Albumin

		Mean	SD	
	n	mg/L		CV, %
Within run	19	132.5	6.0	4.5
		59.7	1.6	2.6
		29.3	0.7	2.3
Between day	32	125.0	6.1	4.8
(calibrating on day 1)		60.0	3.9	6.6
		30.0	2.1	7.0
Between day	32	126.4	5.7	4.5
(calibrating on each day)		59.1	2.9	4.9
		27.7	2.7	9.7

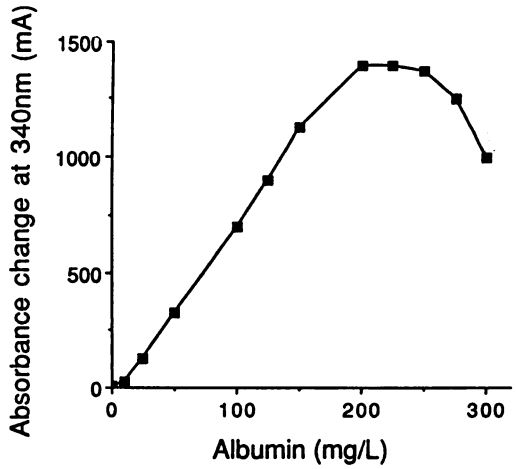

Fig. 1. Urinary albumin standard curve, measured over 4 min
An absorbance change greater than that for the 100 mg/L standard is obtained, even with a sample concentration of 300 mg/L

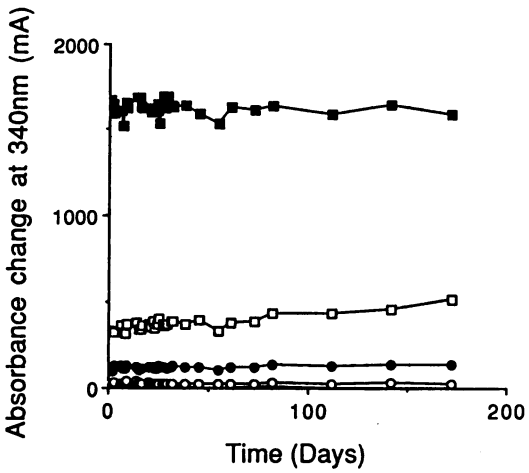

Fig. 2. Variation in the change of absorbance for each calibrant during six months (○ 0, ● 20, □ 50, ■ 200 mg/L)
For all calibrants CVs of absorbance changes were <10%

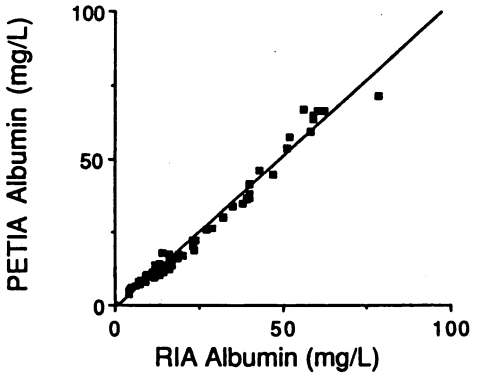

Fig. 3. Method comparison for urinary albumin concentration as measured by PETIA and Pharmacia RIA
Regression analysis showed PETIA = 1.04 RIA − 1.25 (r = 0.99)

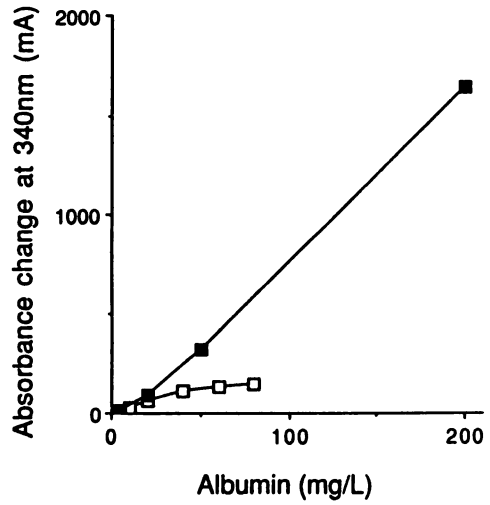

Fig. 4. Comparison of (■) latex-enhanced and (□) nonenhanced immunoturbidimetric assays for urinary albumin, utilizing the same antibody

styrene shell. This core material possesses a higher refractive index than that of many previous latex particles, providing an opportunity for enhanced sensitivity. The chloromethylstyrene shell enables proteins to be covalently coupled to the latex particles simply and reproducibly, with 40-nm particles giving a lower detection limit than larger particles. The smaller size also favors the maintenance of a homogeneous reagent mixture that does not sediment or self-aggregate.

The stability of covalent coupling of antibody to particle was demonstrated by Price et al. (*17*) in a rapid assay for C-reactive protein; the working reagent, and hence calibration curve, were found to be stable for at least 16 weeks. Our albumin assay confirms this, because precision and calibration curve stability were easily maintained during the six months studied. Most analyzer systems are now capable of storing calibration-curve data and therefore this stability has many practical benefits, including decreased reagent and calibration material usage together with faster delivery of results.

The assay presented here for albumin in urine is precise, and results compare well with those by an established RIA. The detection limit in the presented assay is 2 mg/L, but it can be decreased further to 0.5 mg/L or less by increasing the sample volume and re-optimizing the assay. This assay covers a wide range of albumin concentrations, 5 to 200 mg/L, and achieves a far greater signal than does the direct (nonenhanced) immunoturbidimetric assay (Figure 4). This increased sensitivity results in superior precision and the opportunity to maintain good analytical performance with use of less-sophisticated photometric equipment. This type of assay can therefore be readily adapted for use outside the laboratory—for example, with use of a hand-held microtiter-well strip reader.

Viberti and Vergani (*18*) described a simple direct latex-agglutination assay that they claimed gave a "positive answer" in the range 25–166 mg/L. Paoli et al. (*19*) described an indirect latex-agglutination assay designed to give a cutoff (negative result) at an albumin concentration of ≤40 mg/L. Paoli et al. used simple visual detection of agglutination and argued that, by use of an indirect method in which albumin was coupled to latex beads, the possible error due to antigen excess was eliminated. However, these authors were unable to produce a quantitative result with their method.

Samples with albumin concentrations >200 mg/L can readily be detected with a simple stick test (e.g., Albustix; Miles-Ames, Elkhart, IN) so that necessary dilutions can be made before analysis with our immunoassay. Additionally, most automated instruments for which this assay is appropriate have algorithms for detecting antigen excess to circumvent this problem.

We calculated recoveries for both centrifuged and noncentrifuged urine samples stored at 4 °C, because the literature seems confused as to the effect, if any, of centrifugation on the measurable albumin concentration after storage at 4 or −20 °C for any substantial interval (*20, 21*). We found a limited advantage with centrifugation, and we recommend it to ensure removal of any endogenous matter that may be present after cold storage of samples.

In an assessment of immunochemical methods for the determination of albumin in urine, Watts et al. (*22*) stated that the preferred method must be sensitive, specific, and practicable. Practicality can be extended beyond the requirements of the laboratory to encompass use in the outpatient clinic. If an assay is sufficiently rapid and robust such that an accurate result can be produced in 5 min by nontechnical personnel, then the screening of all diabetics during a clinic visit becomes attractive and cost-effective.

References

1. Carone FA, Peterson DR, Oparil S, Pullman TN. Renal tubular transport and catabolism of proteins and peptides. Kidney Int 1979;16:271–8.
2. Sumpio BE, Hayslett JP. Renal handling of proteins in normal and disease states [Review]. Q J Med 1985;57:611–35.
3. Mogensen CE, Christensen CK. Predicting diabetic nephropathy in insulin dependent patients. N Engl J Med 1984;311:89–93.
4. Viberti GC, Pickup JC, Jarrett RJ, Keen H. Effect of control of blood glucose on urinary excretion of albumin and β_2-microglobulin in insulin-dependent diabetics. N Engl J Med 1979;300:638–41.
5. Woo J, Floyd M, Cannon DC, Kahan B. Radioimmunoassay for urinary albumin. Clin Chem 1978;24:1464–7.
6. Fielding BA, Price DA, Houlton CA. Enzyme immunoassay for urinary albumin. Clin Chem 1983;29:355–7.
7. Silver A, Dawnay A, Landon J, Cattell WR. Immunoassay for low concentration of albumin in urine. Clin Chem 1986;32:1303–6.
8. Teppo AM. Immunoturbidimetry of albumin and immunoglobulin G in urine. Clin Chem 1982;28:1359–61.
9. Harmoinen A, Vuorinen P, Jokela H. Turbidimetric measurement of microalbuminuria. Clin Chim Acta 1987;166:85–9.
10. Litchfield WJ, Craig AR, Frey WA, Leflar CC, Looney CE, Luddy MA. Novel shell/core particles for automated turbidimetric immunoassays. Clin Chem 1984;30:1489–93.
11. Price CP, Spencer K, eds. Centrifugal analysers in clinical chemistry. Eastbourne, U.K.: Praeger, 1980.
12. Price CP, Spencer K, Whicher J. Light scattering immunoassay of specific proteins: a review. Ann Clin Biochem 1983;20:1–14.
13. Cooper AG, Brown MC. Automated hemagglutination inhibition. In: Ritchie RF, ed. Automated immunoanalysis, Part 2. New York and Basel: Marcel Dekker Inc., 1978:471–506.
14. Lamont-Havers RW. Nature of serum factors causing agglutination of sensitized sheep cells and group A hemolytic streptococci. Proc Soc Exp Biol Med 1955;88:35–8.
15. Leuvering JWH, Thal PHJM, Van der Waart M, Schusso AHWM. Sol particle immunoassay (SPIA). J Immunoassay 1980;1:77–91.
16. Galvin JP. Particle enhanced immunoassays—a review. In: Diagnostic immunology: technology assessment and quality assurance. Skokie, IL: College of American Pathologists, 1983:18–30.
17. Price CP, Trull AK, Berry D, Gorman EG. Development and validation of a particle-enhanced immunoassay for C-reactive protein. J Immunol Methods 1987;99:205–11.
18. Viberti GC, Vergani D. Detection of potentially reversible

diabetic albuminuria: a three drop agglutination test for urinary albumin at low concentration. Diabetes 1982;31:973–5.
19. Paoli C, Bardelli F, Tarli P, Gragnoli G, Tanganelli I, Signorini AM. A simple latex agglutination test for urinary albumin screening [Brief Tech Note]. Clin Chim Acta 1987;166:67–71.
20. Townsend JC. Specimen preparation for microalbuminuria assay [Tech Brief]. Clin Chem 1986;32:559.
21. Silver AC, Dawney A, Landon J. Specimen preparation for assay of albumin in urine [Letter]. Clin Chem 1987;33:199–200.
22. Watts GF, Bennett JE, Rowe DJ, et al. Assessment of immunochemical methods for determining low concentrations of albumin in urine. Clin Chem 1986;32:1544–8.