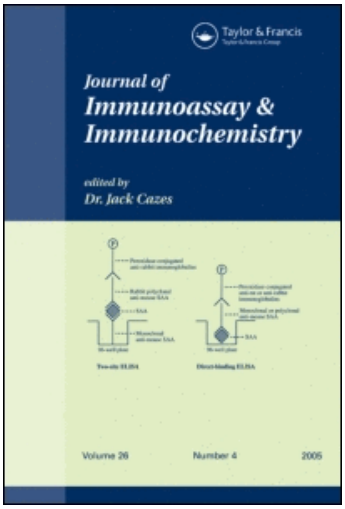

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

The Development and Validation of a Competitive, Microtiter Plate Enzymeimmunoassay for Human Albumin in Urine

Serif Akman[a]; Ismail Kurt[a]; Mustafa Gultepe[a]; Ilker Dibirdik[a]; Cumhur Kilinc[a]; Turker Kutluay[a]; Levent Karaca[a]; Namik Kemal Bingol[a]
[a] Department of Biochemistry, School of Medicine, Gulhane Military Medical Academy, Ankara, Turkey

To cite this Article Akman, Serif , Kurt, Ismail , Gultepe, Mustafa , Dibirdik, Ilker , Kilinc, Cumhur , Kutluay, Turker , Karaca, Levent and Bingol, Namik Kemal(1995) 'The Development and Validation of a Competitive, Microtiter Plate Enzymeimmunoassay for Human Albumin in Urine', Journal of Immunoassay and Immunochemistry, 16: 3, 279 — 296
To link to this Article: DOI: 10.1080/15321819508013563
URL: http://dx.doi.org/10.1080/15321819508013563

JOURNAL OF IMMUNOASSAY, 16(3), 279-296 (1995)

THE DEVELOPMENT AND VALIDATION OF A COMPETITIVE, MICROTITER PLATE ENZYMEIMMUNOASSAY FOR HUMAN ALBUMIN IN URINE

Serif Akman, Ismail Kurt, Mustafa Gultepe, Ilker Dıbırdık
Cumhur Kılınc, Turker Kutluay, Levent Karaca, Namik Kemal Bingol
Department of Biochemistry, School of Medicine,
Gulhane Military Medical Academy, Ankara, Turkey

ABSTRACT

We have described a fast, simple and sensitive microtiter scale, solid phase, competitive enzymeimmunoassay (EIA) for the determination of urinary albumin. The albumin used in the test system was purified by the combination of PEG precipitation and DEAE-cellulose column chromatography. In this EIA, microtiter plates were coated with rabbit antihuman albumin IgG, and incubated with HRP-albumin conjugate with either sample or standards. O-phenylenediamine (OPD) and H_2O_2 solution was used as substrate for HRP. Results obtained correlate well (r= 0.994) with those of an in-house RIA in which same antibody and standards were used as in EIA. The present assay covers the range of 0.5 to 10 mg/L and can be performed in 2 hours. The detection limit was 0.15 mg/L of albumin. Within - assay coefficient of variation was 8.1% and 6.6% and between - assay variation was 10.6% and 8.6% at 1.25 and 2.5 mg/L respectively.

(KEY WORDS: EIA, RIA, Microalbuminuria, albumin purification.)

INTRODUCTION

Sensitive assays for urine albumin are used for the measurement of levels of protein which are above the upper normal limit, but not detectable by standard clinical tests such as albustix (Miles GmbH, Sparte Ames, Frankfurter, West Germany).

Laboratories are required to measure these low concentrations of albumin in urine since this slight albuminuria may be associated with the development of diabetic nephropathy. Various methods have been described for quantifying low concentrations of albumin in urine including radial immunodiffusion (1) and "rocket" immunoelectrophoresis (2). More recently, sensitive radioimmunoassays (RIA) have been described for quantifying albumin in urine (3, 4, 5). Other assays developed for urine albumin determinations are Latex-bead immunoagglutination (6, 7), turbidimetric immunoassay (8, 9, 10, 11), particle enhanced turbidimetric immunoassay and dye - binding methods (13, 14, 15). In 1983, Fielding et al. (16) documented a "sandwich" - type enzyme linked immunosorbent assay for albumin overcoming the disadvantages of short shelf life, health and safety hazards and equipment expense associated with RIA. Here we describe a purification method for human albumin, and a simple competitive enzymeimmunoassay in which HRP - conjugated human albumin and albumin compete for a limited amount of anti-albumin antibody coated to microtiter plates. This procedure has advantages over other techniques and is easily set up. We have also assessed this assay by comparison with a "in house" RIA.

MATERIALS AND METHODS

Apparatus

A UV spectrophotometer (Shimadzu graphicord UV - 240, Japan) and multihead gamma counter (Model NE 1612; Nuclear Enterprises Ltd, Edinburgh, Scotland, U. K.) were used. An ELISA processor II (Behring, Marburg F. R. G.) was used for all

washing, reagent dispensing and absorbance reading procedures. Nunc certificate grade 96 - well microtiter plates used were from Inter-med, Kamstrup, Denmark. The column chromatograpy system with fraction collector and UV - Cord detector, was from LKB, Sweden. All filtration material and concentrator were from Sartorious GmbH, West Germany. Immune and cellulose acetate electrophoresis system were from Pharmacia Ltd. , Milton Keynes, U. K. A refrigerated centrifuge used was from IEC , Damon, U.S.A. (Model, B 20 A).

Reagents

Human serum albumin (HSA) was purified in our laboratory and used as immunogen, as calibrator, and for iodination. Bovine serum albumin (BSA), horseradish peroxidase (HRP), o-phenylene diamine (OPD), glutaraldehye (GA) were purchased from Sigma Chemical Co, St Louis., USA. Sephadex G - 25, sephadex G-200, DEAE - cellulose were obtained from Pharmacia, Milton Keynes, Bucks., U. K. Antisera raised in rabbit against human albumin was prepared in our laboratory. Other chemicals were obtained from Merck Darmstadt, Frankfurter, W. G. $Na^{125}I$ was from Amersham International Plc. , Amersham, Bucks. U. K.

Buffers and Standards

Coating solution, pH 9.6: 8.5 g of NaCl, 1.0 g of gelatin, and 0.2g of NaN_3 were dissolved in 1 L of distilled water. The pH was adjusted to 9.6 with 5 mol/L NaOH solution. This reagent is stable for 5 months, when stored at 4^0C.

Washing solution and assay diluent, pH 7.4:8 g of NaCl, 0.2 g of KH_2PO_4, 2.8 g of Na_2HPO_4. $12H_2O$ and 0.2 of sodium azide were dissolved in 1 L of distilled water and 500 µg of Tween 20 (polyoxyethylene sorbitan mono laurate) was added as dispersing agent. This solution was used as washing solution. The same solution plus 1 g of gelatin per liter was the assay diuent.

Electrophoresis buffer, pH 8.6: 7.5 g of Tris, 10 g of sodium hippurate, 3 g of hippuric acid and 0.1 mg of sodium azide were dissolved in 1 L of water.

Citrate buffer, pH 5.0 (0.1 M/L): 2.58 g Tri sodium citrate dihydrate is dissolved in 100 ml of distilled water. The pH adjusted to 5.0 with concentrated HCl.

Stock phosphate buffer, 0.5 M, pH 7.4: 19.5 g of sodium dihydrogen phosphate dihydrate and 188.2 g of disodium hydrogen phosphate dodecahydrate were dissolved in 1 L of water. Other phosphate buffers were prepared from this buffer by dilution.

Substrate solution: This was prepared immediately before use by adding 6 µl of 30% hydrogen peroxide (H_2O_2) solution to 10 mg of OPD in 20 ml of citrate buffer. The substrate solution was prepared in and dispensed from a brown bottle.

Standards: Human albumin purified in our laboratory was calibrated against SPS - 01, a calibration material for specific protein assay from the supraregional specific protein reference unit of the Royal Hallamshire Hospital, Sheffield, U. K. , and diluted in the assay diluent to give a stock solution of 200 mg/L. This stock standard

was stored in aliquots at - 20°C. From this, standards were made up in diluent at concentration of 0.16, 0.32, 0.64, 1.25, 2.5, 5, 10, 20, 50 mg/L. Fresh standards were prepared every three days.

Urine Samples

Untimed midstream urine samples were collected from apparently healthy hospital personnel and urine samples were obtained from albumin - test - strip negative patients with type I and type II diabetics and stored at - 20°C.

Purification of Human Albumin

5 ml of PEG 6000 in a 25% (w/v) water solution (pH 7.0) was added to 5 ml of serum with stirring to make a final PEG concentration in serum of 12 % w/v. The mixture was stirred at 4°C for 60 min. and the precipitate was removed by centrifugation at 2000 g for 15 min. The supernatant was adjusted to pH 4.6 using 1 M HCl and solid PEG 6000 was added bringing the final PEG concentration to 25% (w/v). The mixture was again stirred for 60 min. at 4°C and the albumin containing precipitate was collected by centrifugation at 2000 x g. To remove residual immunoglobulins, the precipitate was washed with a volume of 12% PEG 6000 solution (pH 7.0) corresponding to 70% of the final volume of 8% albumin solution and the precipitate was discarded by centrifugation. The pH of the supernatant was adjusted to 5.2 using 10 M HCl in order to remove residual PEG. The albumin was precipitated from the supernatant by ethanol (40%) at - 5°C. The concentration of

albumin in the precipitate was adjusted to 8 mg/ml in 10 mM phosphate buffer, pH 6.8 and applied to a column of DEAE - cellulose previously equilibrated in the same buffer (17). Between 10 and 15 ml of packed DEAE-cellulose were used per ml of serum to be fractioned. After sample application the column was washed with the equilibration buffer, and lgG which does not bind to the adsorbent under these conditions, washed through the column. The buffer pH was lowered to pH 4.6 (50 mM sodium acetate - acetic acid buffer) and the ionic strength increased to 0.07, thus eluting the albumin (Figure 1). Remaining protein was washed off the column at pH 4.0 I = 0.15. The ion exchanger was then regenerated in the column by washing with two column volumes of 10 mM phosphate buffer pH 6.8 after which a new sample could be applied.

The purity of the final preparations of albumin were checked with cellulose or immunoelectrophoresis in which rabbit anti-human albumin was used as antibody. Only a single band corresponding to albumin was obtained (result not shown). The concentration of agarose in the immunoelectrophoresis plate was 1%. The albumin preparations (10 µl) were electrophoresed at a current of 2-3 mA/cm for 90 min. The plate was washed and antihuman albumin antiserum placed in a trough cut in the gel and put into the humid chamber overnight. The antiserum diffused into the gel, meeting the electrophoresed albumin as a single arc formed by insoluble antigen-antibody complexes. The purity of the preparations of albumin were verified by testing them with simple immunodiffusion. Three different antihuman albumin antisera (sheep and rabbit obtained from Sigma Chemical Co. and home-made

respectively) and antisera against serum proteins anti-human IgG, IgM, anti-ferritin, and whole antiserum against human serum were placed in a succession of Ouchterlony wells in 1% agar gel contained in a petri dish. Albumin preparation at a concentration of 1 mg/ml was placed in the central well. The plate was covered and left in a humid environment for 48 h. A precipitin line between albumin and antisera raised against albumin were formed. There were no precipitin lines between albumin and the antisera against other serum proteins.

Production of Antiserum

Antibody against human albumin was obtained with 10 mg of human albumin in one ml water emulsified with one ml of complete Frund's adjuvant and injected subcutaneously into five rabbits at weekly intervals for five weeks. A satisfactory titer of antibody was obtained in three animals. The titer of the antialbumin was determined in a dilution curve. The antibodies obtained in satisfactory titer were applied to human serum immunelectrophoresis (electrophoresis buffer, pH 8.6) to check the specificity and quality. Only a single band corresponding to human albumin was obtained.

Preparation of Immunoglobulin Fraction

3600 mg of anhydrous Na_2SO_4 powder was added, with stirring, to 20 ml rabbit anti-albumin antiserum resulting in an 18% (w/v) solution. After five minutes continous stirring at room temperature, 5 ml of 18% Na_2SO_4 (w/v) was added and centrifuged (3000 x g, 10 min). The supernatant was discarded and the precipitate was

reconstituted in 20 ml of 0.15 M NaCl. The same procedure was repeated as described above and the last precipitate was reconstituted in 6 ml of 0.15 M NaCl (30 - 40% of original serum volume) and stored at - 20°C in aliquots.

Preparation of ^{125}I-Labelled Albumin

16.5 mg albumin was iodinated with 1 mCi of $Na^{125}I$ by the chloramine T method (18). The reaction mixture was applied to a 1 x 36 cm column of sephadex G-200 eluted with assay diluent, and collected in fractions. The radioactivity of these fractions were measured and the principal albumin peaks were pooled to give the stock tracer preparation. Aliquots stored at - 20°C.

Conjugation of HRP to Albumin

HRP was conjugated to albumin by the two-step glutaraldehyde method as described by Avrameas (19). Briefly 5 mg of HRP was suspended in 0.2 ml of 0.1 M phosphate buffered saline (PBS), pH 6.8, containing 1.25% glutaraldehyde. After an overnight incubation at 22°C, the mixture was dialysed against normal saline and adjusted to a volume of 1 ml with saline. One ml of a 2.5 mg/ml albumin saline solution and 0.1 ml of 1 M carbonate buffer pH 9.5, were added to the activated HRP solution and incubated at 4°C for 24 h. After addition of 10 µl of a 0.2 M solution of lysine, the mixture was incubated at room temperature for 2 h and dialysed overnight at 4°C against 0.05 M PBS pH 7.2. Large conjugates were removed by centrifugation for 30 min at 20,000 x g and the supernatant was stored at 4°C in the dark.

EIA Procedure

200 µl of rabbit anti-albumin IgG fraction in coating solution were incubated overnight at 4°C in all but two wells of a polystyrene microtiter plate. The plates were washed three times with washing solution, and 50 µl of samples or standards were added with 100 µl of HRP - albumin conjugate (1/10,000 dilution in assay diluent) to the wells. After 1 hour incubation at 37°C the plate was washed again three times with washing solution. 100 µl of freshly prepared substrate solution was applied to all the wells and after incubation in the dark at room temperature for 20 minutes, the reaction was terminated by adding 200 µl of 4 N H_2SO_4. 200 µl of substrate solution was used in the two uncoated wells as blank. The concentration of albumin in the test urines were deduced by reference to a standard curve.

RIA Procedure

All urine samples were centrifuged at 3000 g for 10 minutes at 4°C prior to albumin determination. 100 µl of urine or standards was added to a premixed of 100 µl of ^{125}I - Albumin (12,000 cpm, 2.5 ng in assay diluent, specific activitiy of ^{125}I- Albumin was 140 µCi /µg) and 100 µl of rabbit anti-albumin IgG (1/160,000 dilution in assay diluent) vortex-mixed and incubated at room temperature for 2 hours. Precipitation of bound albumin was achieved by adding 200 µl of horse serum, diluted 1/2 in saline and 500 µl of PEG 6000 solution (20% w/v in water) to each tube followed by mixing. After incubation at room temperature for 1/2 hour, centrifugation and aspiration of supernatant were carried out. The precipitate was counted in a

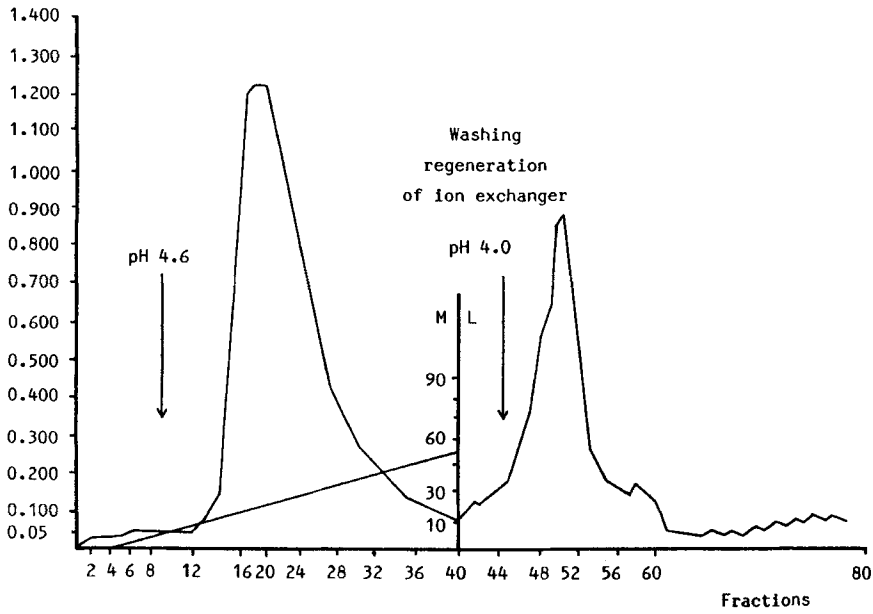

Figure 1: The elution profile of albumin from a DEAE - cellulose column. The column was run at 8°C with phosphate buffer (10 mmol/L, pH 6.8) in an ascending manner at 100 ml/h flow rate. The peak of monomeric human albumin (first peak) was eluted with a linear gradient consisting of 100 ml each of 10 mmol/L and 100 mmol/L acetate buffer. The remaining protein was washed off the column at pH 4.0.

ɣ - counter for 1 minute. The standard curve was plotted as the % tracer bound vs. albumin concentration. Unknown values are determined from this curve.

RESULTS

Purification of Human Serum Albumin

Human serum albumin was separated with PEG 6000 and applied to a DEAE - cellulose column. The optimum conditions required for the two steps were investigated and the effects of pH, ionic strength and temperature were examined. The

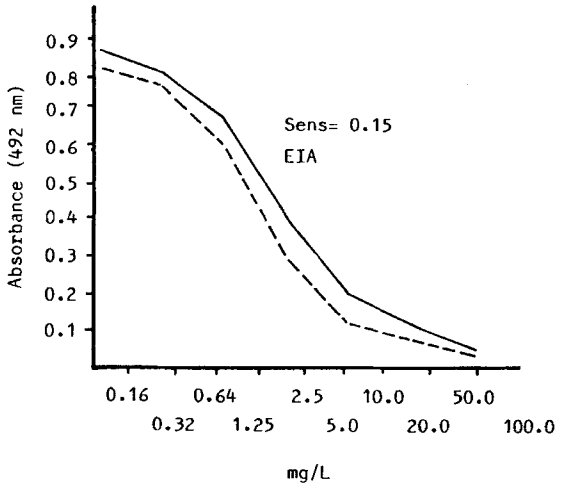

Figure 2: The standard curves of RIA (----------) and EIA (————) of albumin. The nonspecific binding has been subtracted.

purity of final preparations of albumin were checked with immunoelectrophoresis in which rabbit antihuman albumin was used as antibody. Only a single band corresponding to albumin was obtained (result not showed). The elution profile of albumin from a DEAE-cellulose column was shown in Figure 1. The albumin concentration were determined from spectrophotometry at 280 nm, using an absorptivity of 0.54 for a 1 mg/ml solution of human albumin. With this purification method, a yield of 2.3 g of albumin with a purity of 97% was obtained from each 100 ml of serum.

Characteristics of EIA

Figure 2 shows the standard curves of RIA an EIA of albumin. The nonspecific binding has been subtracted from all points but was always less than 2% in RIA and 3.5% in EIA.

The detection limit calculated from the standard deviation of absorbances for 20 replicates of the zero concentration standard at Bo-(2xSD) was 0.15.

Specificity: Proteins that may be present in urine were dissolved in diluent buffer to give concentrations greater than would be encountered in clinical practice. When we tested them in EIA we found no effect of human β2 - microglobulin on the assay at 200 mg/L, IgG at 10 g/L, or ferritin 0.5 g/L. Bovine serum albumin had a 20% cross reactivity in this assay.

Analytical recovery: Recovery of human albumin added to give incremental albumin concentrations of 2, 5, 10, 20 and 50 mg/L to each of six urine samples with albumin concentrations between 10 and 60 mg/L, ranged from 93 to 115%, with a mean of 101%.

Assay parallelism: Three urine samples with albumin concentrations in the normal and pathological range gave curves paralleling that for the human albumin standards when the samples were serially diluted.

Imprecision: Within assay precision profile was determined from results for 10 duplicates of each assay standard (Figure 3). Between assay precision, calculated from results for urine pools assayed in duplicate in 10 consecutive assays was 15% at 0.3 mg/L, 10% at 1.2 mg/L and 8% at 2.5 mg/ L.

Assay range: The assay range determined from the precision profile (Figure 3) was from 0.5 to 12 mg/L.

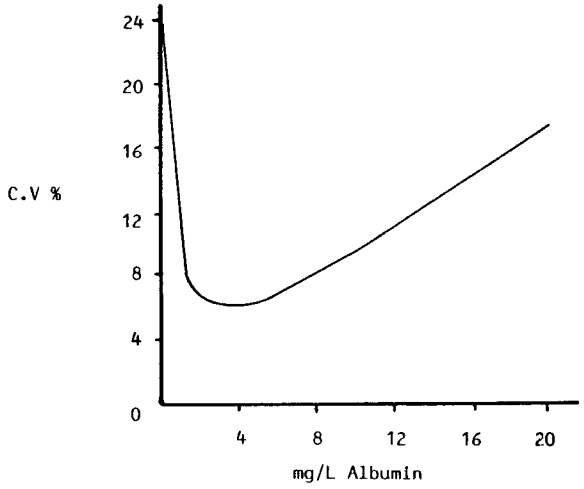

Figure 3: Within assay precision profile of albumin EIA.

Effects of osmolality, glucose and pH: Glucose (100 mmol/L), urea (2 mol/L), sodium chloride (1 mol/L) solutions caused no significant change in HRP - conjugate binding as compared with the zero - concentration standard.

Storage of urine samples: Samples stored at 4°C, or frozen at - 20°C for 30 days showed no change in assayed concentrations. Freezing and thawing five times also had no effect (results not shown).

Correlation of EIA and RIA: Our in-house RIA was previously compared with the RIA of a commercial company (DPC, USA). A good correlation was between these two methods. The in-house EIA was compared with our RIA. Using both protocols, we assayed 93 urine specimens (range of albumin concentrations 0.1-22.2 mg/L), from healthy subjects and albumin test-strip negative urine samples from patients who

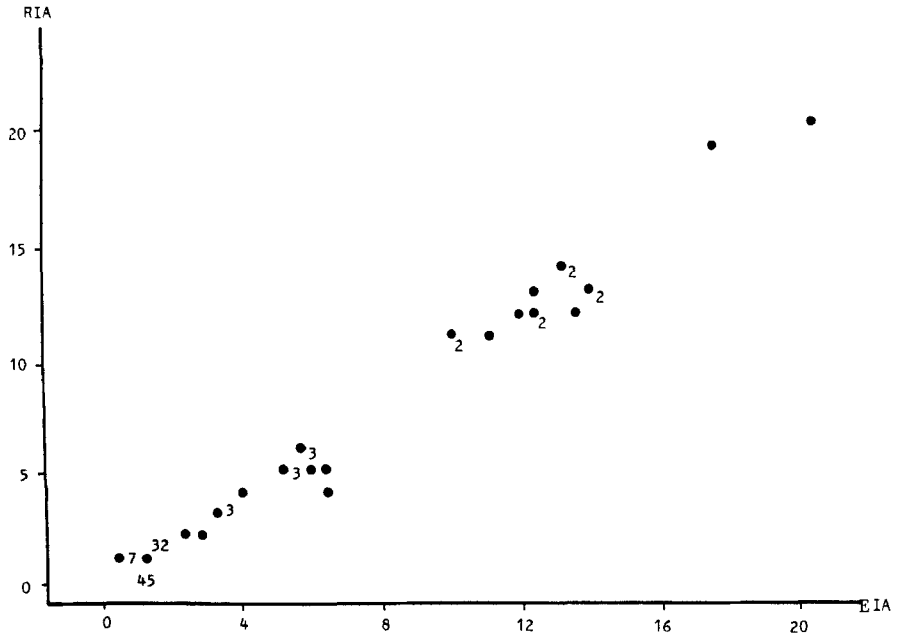

Figure 4: Correlation of EIA and in house RIA in which same antihuman IgG and standards have been used.

were attending a diabetes clinic. Correlation between the two assays was good ($r= 0.99$, $p<0.01$), (Figure 4).

Reference interval: The normal range for urinary albumin based on 125 healthy laboratory subjects was 2.2 - 12.6 mg/L.

DISCUSSION

In this study, a simple separation and one step chromatographic purification process was developed for human albumin from serum. This albumin was used to develop an immunoassay. Before precipitation of albumin at the isoelectric point

(pH 4.5) with a mild agent, PEG 6000 (25%), globulins were removed at pH 7.0 with 12% PEG 6000. PEG is a nontoxic, water soluble synthetic polymer whose use as a precipitating agent was first reported by Albertsson (21). The main advantage of PEG over ethanol or salt precipitation is the shorter time required for the precipitation process to reach equilbrium (22). Moreover, PEG has a very low heat of solution in water so that large temperature increases do not accompany addition of the reagent. Residual PEG was easily removed from albumin preparations by the precipitation of albumin with ethanol. The result was satisfactory, as far as yield and purity are concerned, comparing with those obtained before (17, 21, 22).

In this EIA urine need not be diluted before the assay. The range of our assay was derived from the precision profile shown in Figure 3, using a cut-off of 10% CV. The range can extend to nearly 20 mg/L with some loss of precision. By using precoated plates, an entire assay - including sample preparation, reagent addition, incubation and end point determination may be completed within 2 hours. On the other hand, existing immunoassays involve either sample dilution before assay (23), use of an unusually small sample volume (24) or long incubation times (25) and time consuming separation techniques. Moreover, in most studies of this kind, timed overnight or 24 h urine specimens were used, which are difficult to collect accurately, and are inconvenient for patients. So we have chosen to base our reference interval on random, daytime midstream urine samples. If the result is abnormal the test can easily be repeated or followed up with a timed collection.

To overcome high, nonspecific binding and poorly reproducible results and avoid possible cross-reactivity between human and bovine serum albumin, we tried Tween 20 and gelatin in the diluent/washing solution as suggested by Kramer et al, (26) to improve sensitivity of the test.

The precision of the method is comparable with other methods (5, 24). The agreement between EIA method and our in-house RIA is good (Figure 4).

In addition, use of the rabbit antihuman albumin IgG fraction enabled lower concentrations of albumin to be distinguished without either the loss of response or limitation of assay range that is encountered when antiserum is directly used.

Although the assay range (0.5 - 12 mg/L) and sensitivity (0.15 mg/L) for this competitive method is less than reported by Fielding et al. (16) (range 3 - 1000 µg/L sensitivity 0.5 µg/L), the slightly reduced sensitivity and range are not a practical problem. Because urine dilutions show parallelism, the effective range of the assay may be adjusted to suit a particular application.

As a result, this method is suitable for the rapid and inexpensive screening of large numbers of urine samples for small increases in albumin concentration.

Correspondence: Dr. Şerif AKMAN

Department of Biochemistry, School of Medicine, Gulhane Military Medical Academy, Etlik-Ankara, Turkey.

REFERENCES

1. Mancini G, Carbonara A D, Heremans J F. Immunochemical quantitation of antigens by single radialimmunodiffusion. Immunochemistry 1965; 2 : 235 - 9.

2. Laurel C B, Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 1969; 15; 45 - 9.

3. Miles D W, Mogensen C E, Gundersen H J G. Radioimmunoassay for urinary albumin using a single antibody. Scand. J. Clin. Lab. Invest. 1970; 26: 5 - 11.

4. Woo J, Floyd M, Cannon D C, Kahan B. Radioimmunoassay for urinary albumin. Clin. Chem. 1978; 24: 1464 - 7.

5. Silver A, Dawney A, Landon J, Cattell W R. Immunoassays for low concentrations of albumin in urine. Clin. Chem. 1986; 32: 1303-6.

6. Viberti G C, Vergani D. Detection of potentially reversible diabetic albuminuria: a three drop agglutination test for urinary albumin at low concentration. Diabetes 1982; 31: 973-5.

7. Hodgson B W, Watts G F. A simple side room test to screen for "microalbuminuria" in diabetes mellitus. Ann. Clin. Biochem. 1987; 24: 581-4.

8. Teppo A M. Immunoturbidimetry of albumin and immunoglobulin G in urine. Clin. Chem. 1982; 28: 1359 - 61.

9. Sathianathan P, Rege U P, Barron J L. Immunoturbidimetric determination of urinary albumin with a centrifugal analyzer (Letter). Clin. Chem. 1986; 32: 202.

10. Watts G F, Bennet J E, Rowe D J et al. Assessment of immunochemical methods for determining low concentrations of albumin in urine. Clin. Chem. 1986; 32 : 1544-57.

11. Biou D, Therond P, Israel A, Demelier J F. Rapid assessment of albumin concentration by immunoturbidimetry. Clin. Chem. 1985; 4: 620 - 1.

12. Medcalf E A, Newman D J, Gorman E G, Price C P. Rapid, robust method for measuring low concentrations of albumin in urine. Clin. Chem. 1990; 3: 446 - 9.

13. Jung K, Nickel E, Pergande M. A microalbuminuria assay using bromphenol blue. Clin. Chim. Acta. 1990; 187: 163 - 72.

14. Philipou G, James S K, Seaborm C J, Phillips P J. Screening for microalbuminuria by use of a rapid, low cost colorimetric assay. Clin. Chem. 1989; 3: 456 - 8.

15. Schosinsky K H, Vargas M, Esquirel A L, Chavarria M A. A simple spectrophotometric determination of urinary albumin by dye - binding with use of bromphenol blue. Clin. Chem. 1987; 2: 223 - 6.

16. Fielding B A, Price D A, Houlton C A. Enzymeimmunoassay for urinary albumin. Clin. Chem. 1983; 29: 335 - 7.

17. Curling J M, Berglöf J, Lindquist L O, and Ericksson S. A chromatographic procedure for the purification of human plasma albumin. Vox Sang. 1977; 33: 97 - 107.

18. Hunter W M, Greenwood F C. Preparation of iodine - 131 labelled human growth hormone of high specific activity. Nature (London) 1962; 194: 495 - 6.

19. Avrameas S, Ternynck T. Peroxidase labelled antibody and Fab conjugates with enhanced intracellular penetration. Immunocytochemistry 1971; 8: 1175 - 9.

20. Rodbard D. Statistical estimation of the minimal detectable concentration ("sensitivity") for radioligand assays. Anal. Biochem. 1978; 90: 1 - 12.

21. Albertsson P A. Partition of cell particles and macromolecules. 1 st ed. New York: J., 1960; 154.

22. Foster P. Proceedings of the International workshop of technology for protein separation and improvement of blood plasma fractionation. Washington D. C: U. S. Govt. Printing Office, 1978.

23. Nargessi R D, Landon J, Pourfarzaneh M, Smith D S. Solid phase fluoroimmunoassay of human albumin in biological fluids. Clin. Chim. Acta. 1978; 89: 455 - 60.

24. Christiansen C K, Qrskow C. Rapid screening PEG radioimmunoassay for quantification of pathological microalbuminuria. Diabetic Nephropathy 1984; 3: 92 - 4.

25. Keen H, Chlouverakis C, An immunoassay method for urinary albumin at low concentrations. Lancet 1963; ii: 913 - 4.

26. Kramer B K, Jesse U, Ress K. M, Schmuling R M, Risler T. Enzyme linked immunosorbent assay for urinary albumin at low concentrations. Clin. Chem. 1987; 33: 609 - 11.