Pure & Appl. Chem., Vol. 68, No. 10, pp. 1881–1885, 1996.
Printed in Great Britain.
© 1996 IUPAC

Determination of β_2-microglobulin in human serum and urine by latex turbidimetry

F.-Javier Gella and María-José Capdevila

Department of Research and Development. Reagents Division. BioSystems, S.A., Barcelona, Spain.

Abstract A latex turbidimetric method for the measurement of β_2-microglobulin concentration in human serum or urine has been evaluated. The method is based on direct agglutination by β_2-microglobulin in human specimens of latex particles coated with an antibody against β_2-microglobulin. The agglutination is quantified by turbidimetry. The assay range is from 0.28 to 18 mg/L for serum and up to 3 mg/L for urine. The measured signal was linearly related to the β_2-microglobulin concentration for the serum assay but it was not for urine. The zone effect was out of physiological β_2-microglobulin concentration. Intra-assay and inter-assay precision were below 10%. Rheumatoid factor (up to 450 IU/mL), bilirubin (1 g/L) and lipaemia (20 g/L) did not interfere. Results of serum samples correlated well with those obtained by a radioimmunoassay method and a nephelometric immunoassay.

INTRODUCTION

The protein β_2-microglobulin is present on the membrane of nearly all nucleated cells, non-covalently linked to the heavy chains of the class I histocompatability antigens. It is a low molecular weight protein (Mr = 11 800) composed of 100 aminoacids (1,2). The protein on the plasma membrane surface is in equilibrium with the surrounding medium, and can be detected at low concentrations in serum (3). Because of its small size, β_2-microglobulin is filtered at the glomerulus and almost totally reabsorbed and catabolised by proximal tubular cells. Circulating concentrations of β_2-microglobulin are influenced by the turnover of nucleated cells, renal function and immune activation, and are a powerful diagnostic and/or prognostic marker in renal disease (4,5,6), autoimmune disorders (7,8), tumoral and proliferative diseases (9,10,11), and acquired immunodeficiency syndrome (12,13). Conversely, increased urinary excretion reflects primarily tubular cell damage (14).

All current assays for β_2-microglobulin are immunoassays. Although its aminoacid sequence has a considerable degree of homology with the constant region of immunoglobulin G, antisera prepared to β_2-microglobulin do not crossreact with the immunoglobulins. The first described methods were single radial immunodiffusion (14) and immunonephelometry (15), but these methods lacked sensitivity for detecting β_2-microglobulin in urine. Highly sensitive radioimmunoassay (16,17) and enzyme immunoassay (18,19) methods were then developed for β_2-microglobulin analysis. However, the disadvantages of these methods (use of radioisotopes, high cost of the kits, time-consuming procedures) stimulated the development of alternative simpler technologies. Particle-enhanced immunoassay was successfully applied to quantifying serum β_2-microglobulin first by Bernard et al (20,21). Latex particles on which an antibody against β_2-microglobulin was adsorbed, agglutinated in the presence of serum or urine β_2-microglobulin. The agglutination was quantified by counting the remaining unagglutinated particles. In a method with a similar principle described by other authors (22,23), the agglutination is quantified by nephelometry. There is also one report in the literature (24) describing a latex-turbidimetric method for the determination of human serum β_2-microglobulin.

The method studied in this report is a latex-turbidimetric assay based on the direct measurement of particle agglutination and suitable for the β_2-microglobulin measurement in both human serum and urine. The method is simple, robust, rapid, economic and with appropriate analytical performance. An additional

advantage is that the assay do not require any special laboratory equipment (nephelometer or particle-counter) because it can be easily adapted to the instrumentation of a standard clinical chemistry laboratory.

MATERIALS AND METHODS

Latex-turbidimetry method: A commercial kit supplied by BioSystems (Barcelona, Spain) was used. The working reagent, prepared as described in the kit insert, consisted in a suspension of latex particles coated with anti-human β_2-microglobulin antibodies in ammonium chloride buffer 0.2 mol/L, sodium azide 0.95 g/L, pH 8.2. Determinations of β_2-microglobulin concentration in serum and urine were manually performed at 37°C on a Uvikon 860 spectrometer (Kontron, Switzerland), or (only in the methods comparison study) on Shimadzu CL-7200 analyser. The latex reagent (1.0 mL) is mixed with 10 µL of serum or with 40 µL of urine and the absorbance is measured at 540 nm after 10 seconds (A_1) and after 3 minutes (serum) or 5 minutes (urine) of the sample addition (A_2). The absorbance difference ($A_2 - A_1$) is proportional to the β_2-microglobulin concentration in the sample.

Comparison methods: For radioimmunoassay (RIA) of β_2-microglobulin we used a commercial kit supplied by Kabi Pharmacia (Uppsala, Sweden). For nephelometry we used the BNA system (Behringwerke, Marburg, Germany). Measurements were carried out following the manufacturer's reagent kit inserts.

Standards: Each method was calibrated using the standards supplied by the respective manufacturer.

Specimens: Human serum samples and urines were obtained from local hospitals. For method comparison, serum specimens were selected covering a wide range of β_2-microglobulin concentrations (1 to 18 mg/L).

Statistical analysis: The relationship between methods were studied using the Passing and Bablock non-parametric method (24).

Other reagents: The following substances were used in the interference studies: bilirubin was from Sigma (USA), Intralipid solution from Boehringer Mannheim (Germany), and rheumatoid factor from BioSystems (Spain).

RESULTS

Calibration curves and prozone

Figure 1 shows typical calibration curves for serum and urine β_2-microglobulin by latex turbidimetry. The serum curve was linear up to 18 mg/L while the calibration curve for urine specimens was slightly sigmoidal in the tested range (0.2 to 3 mg/L). No antigen excess (prozone phenomenon) appears at β_2-microglobulin concentration below 1000 mg/L (Fig. 2). In practice, such high concentrations of β_2-microglobulin in urine or serum have never been observed, so that a prozone effect with unknown specimens is quite unlikely.

Lower detection limit

It was calculated by measuring the obtained signal using saline solution (NaCl 154 mmol/L) as sample in 10 replicate analysis. The obtained mean plus 3 standard deviations was equivalent in the calibration curve to a β_2-microglobulin concentration of 0.28 mg/L.

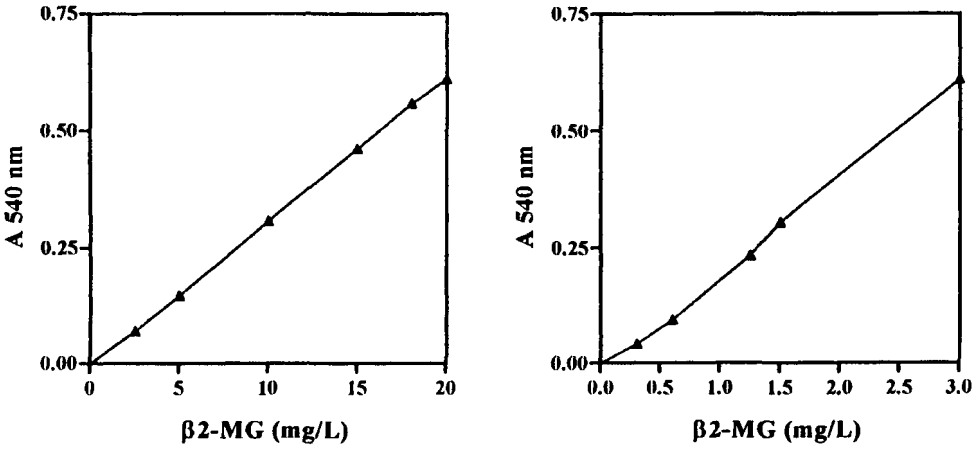

Fig. 1 Calibration curves for serum (left) and urine (right) β_2-microglobulin.

Precision

Intra-assay and inter-assay precision was estimated for 3 different β_2-microglobulin levels. The intra-assay precision was determined by making 20 replicate measurements within a single run. The inter-assay reproducibility was calculated on the basis of 10 determinations of β_2-microglobulin carried out on different days and using a daily calibration curve. Aliquots of the samples were frozen and each aliquot was thawed just before the analysis. Results are shown in Table 1.

TABLE 1. Precision data for the latex turbidimetric method for serum β_2-microglobulin (β2-MG)

β2-MG (mg/L)	CV (%) intra	CV (%) inter
3.5	6.3	8.0
9.5	2.6	3.9
15	3.2	5.7

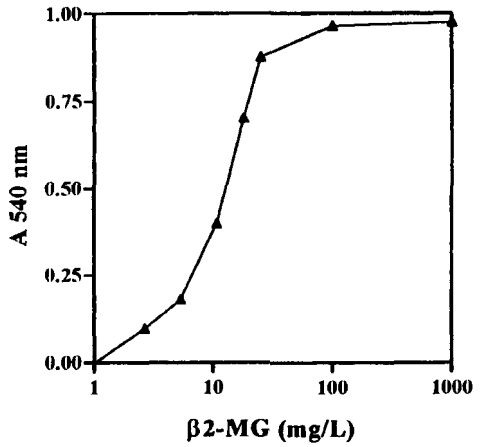

Fig 2. Effect of antigen excess

Interference studies

The effect of bilirubin, lipaemia and rheumatoid factor was studied by adding known amounts of these substances to a serum pool. Rheumatoid factor (up to 450 IU/mL), bilirubin (up to 1 g/L) and lipaemia (up to 20 g/L) did not interfere (data not shown).

Comparison of methods

A good correlation was obtained between the latex turbidimetry and the nephelometric method or the radioimmunoassay (Fig. 3). The obtained values were, however, different as demonstrated by a slope significantly different than 1.0. The observed differences are probably due to the calibration, since different standards (those provided by each manufacturer) were used to calibrate each method.

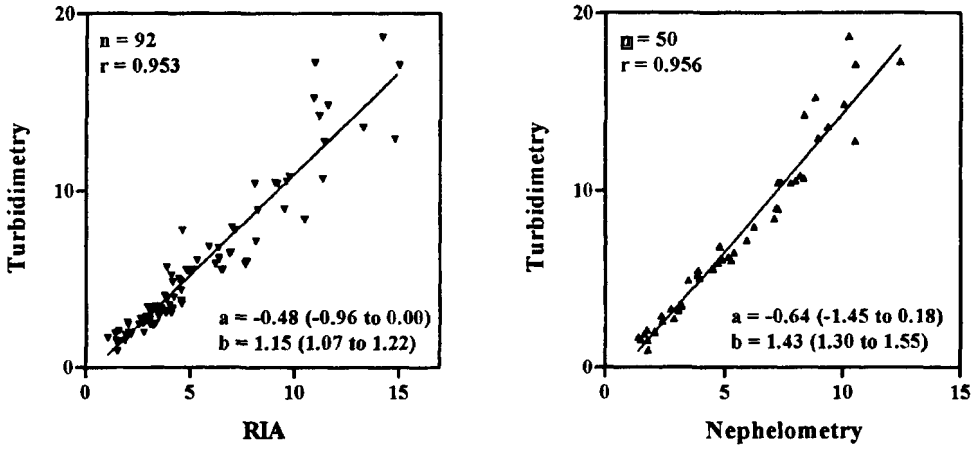

Fig. 3 Comparison of the β_2-microglobulin latex turbidimetric method with the radioimmunoassay (left) and the nephelometric method (right) using human sera. The 95% confidence interval for *a* and *b* is listed in parentheses.

DISCUSSION

Latex agglutination tests have been in use since 1956 to detect a wide range of analytes in the clinical laboratory. Many of the latex agglutination tests developed are performed manually and the agglutination is detected by visual observation. During the last years, several new approaches to detect latex particles agglutination have been described based on the principles of turbidimetry, nephelometry, particle counting, angular anisotropy and quasi-elastic light scattering (25). The great advantage of turbidimetry is that measurements can be made in regular spectrometers and it is easily adapted to the automatic analysers that are commonly found in the clinical laboratories.

Radioimmunoassay and nephelometry are at present the most widely used methods to estimate the β_2-microglobulin concentration in serum or urine samples (23). Although highly sensitive, precise and specific, these methods require special equipment and have an elevated cost. The latex turbidimetric method avoids these disadvantages. Another advantage of the latex turbidimetry is its simplicity: single reagent, no separation steps and it requires an incubation time of only 3 min (5 min for urine samples) while the radioimmunoassay typically incubates for 1 hour and the nephelometric method for 12 min.

The latex turbidimetric method shows analytical characteristics that are similar if not better than that described for the compared methods. The calibration curve was linear up to 18 mg/L while the compared methods show the typical sigmoidal shape common to many immunoassays. This is an important fact because a sigmoidal calibration curve require several standards at different concentrations while the latex turbidimetric method can be calibrated (at least for serum samples) using a single standard. The latex turbidimetric method showed a lower detection limit of 0.28 mg/L. The method has a intra- and inter-assay precision within acceptable limits and it was not interfered by bilirubin, lipaemia and rheumatoid factor. Although the comparison studies with the radioimmunoassay and nephelometric methods showed a good correlation, a bias was observed, with the values obtained by turbidimetry being significantly higher than those by the other methods. However, these differences can be related to the diversity of calibrators used.

In conclusion, the latex turbidimetric method for the measurement of serum or urine β_2-microglobulin is rapid, precise, inexpensive, easily performed (one-step assay), readily adaptable to the clinical laboratory instrumentation, and results correlate well with those by other established methods.

REFERENCES

1. Berggard, L. Bjork, R. Cigen, et al. β_2-Microglobulin. *Scand. J. Clin. Lab. Invest.* **40** (suppl.154), 13-25 (1980).
2. Peterson and L. Rask. Highly purified papain-solubilized HLA antigens contain β_2-microglobulin. *Proc. Natl. Acad. Sci., U.S.A.* **71**, 35-39 (1974).
3. Bernard, A. Byskocil and R.R. Lauwerys. Determination of β_2-microglobulin in human urine and serum by latex immunoassay. *Clin. Chem.* **27**, 832-837 (1981).
4. Wibell, P.E. Evrin and I. Berggard. Serum β_2-microglobulin in renal disease. *Nephron* **10**, 320-331 (1978).
5. Scharddijn and L.W. Statius van Eps. β_2-Microglobulin: its significance in the evaluation of renal function. *Kidney Int.* **32**, 635-641 (1987).
6. Rosenstock and P. Paskin. Early diabetic nephropathy: assessment and potential therapeutic interventions. *Diabetes Care* **9**, 529-545 (1986).
7. Weissel, O. Scherak, H. Fritzsche, et al. Serum β_2-microglobulin and SLE. *Arthritis Rheum.* **19**, 968-971 (1976).
8. Crisp, R.J. Coughlan, D. Mackintosh, et al. β_2-Microglobulin plasma levels reflects disease activity in rheumatoid arthritis. *J. Rheumatol.* **10**, 954-956 (1983).
9. Forman. Serum β_2-microglobulin as an indicator of neoplasia. *J. Clin. Immunoassay* **6**, 228-233 (1983).
10. Hagberg, A. Killander and B. Simonsson. Serum β_2-microglobulin in malignant lymphoma. *Cancer* **51**, 2220-2225 (1983).
11. Bataille, B.M.G. Durie and J. Grenier. Serum β_2-microglobulin and survival duration in multiple myeloma: a simple reliable marker for staging. *Br. J. Haematol.* **55**, 439-447 (1983).
12. Fahey, J.M.G. Taylor, R. Detels, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N. Engl. J. Med.* **322**, 166-172 (1990).
13. Burkes, A.E. Sherrod, M.L. Stewart, et al. Serum β_2-microglobulin levels in homosexual men with AIDS and with persistent, generalized lymphadenopathy. *Cancer* **57**, 2190-2192 (1986).
14. Peterson, P.E. Evrin and I. Berggard. Differentiation of glomerular, tubular and normal proteinuria: Determinations of urinary excretion of β_2-microglobulin, albumin and total protein. *J. Clin. Invest.* **48**, 1189-1195 (1986).
15. Hellsing and H. Enstrom. Pre-treatment of serum samples for immunonephelometric analysis by precipitation with polyethylene glycol. *Scand. J. Clin. Lab. Invest.* **37**, 529-536 (1977).
16. Evrin, P.A. Peterson, L. Wide, et al. Radioimmunoassay of β_2-microglobulin in human biological fluids. *Scand. J. Clin. Lab. Invest.* **28**, 439-443 (1971).
17. Shuster, P. Gold and M.D. Poulik. β_2-Microglobulin in neoplastic diseases. *Clin. Chim. Acta* **67**, 307-313 (1976).
18. Ferrua, C. Vincent, J.P. Revillard, et al. A sandwich method of enzyme immunoassay. III. Assay for human β_2-microglobulin compared with radioimmunoassay. *J. Immunol. Methods* **36**, 149-158 (1980).
19. Bjerrum and H.S. Birgens. Measurement of β_2-microglobulin in serum and plasma by enzyme-linked immunosorbent assay (ELISA). *Clin. Chim. Acta* **155**, 69-76 (1986).
20. Bernard, A. Vyskocil and R.R. Lauwerys. Determination of β_2-microglobulin in human urine and serum by latex immunoassay. *Clin. Chem* **27**, 832-837 (1981).
21. Bernard and R.R. Lauwerys. Comparison of turbidimetry with particle counting for the determination of human β_2-microglobulin by latex immunoassay (LIA). *Clin. Chim. Acta* **119**, 335-339 (1982).
22. Viedma, S. Pacheco and M.D. Albadalejo. Determination of β_2-microglobulin in serum by a microparticle-enhanced nephelometric immunoassay. *Clin. Chem.* **38**, 2464-2468 (1992).
23. Borque, C. Maside, A. Rus and J delCura. Latex immunoassay of β_2-microglobulin in serum and urine. *J. Clin. Immunoassay* **17**, 160-165 (1994).
24. Passing and W. Bablock. A new biometrical procedure for testing the equality of measurements from two different analytical methods. *J. Clin. Chem. Clin. Biochem.* **21**, 709-720 (1983).
25. Gella, J. Serra and J. Gener. Latex agglutination procedures in immunodiagnosis. *Pure & Appl. Chem.* **63**, 1131-1134 (1991).