Influence of Protein Composition on Total Urinary Protein Determined by Pyrocatechol-Violet (UPRO Vitros) and Pyrogallol Red Dye Binding Methods

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Influence of protein composition on total urinary protein assays was evaluated for pyrogallol red-molybdate both with and without sodium dodecyl sulfate (SDS) and pyrocatechol violet-molybdate complex (UPRO) techniques. Using mixtures of albumin and γ -globulins ($n = 8$; albumin/globulin ratio, 0 to 10), mean recoveries were 79, 77, and 81% for pyrogallol red, pyrogallol red-SDS, and UPRO, respectively. Using diluted myeloma sera ($n = 26$; A/G ratio, 0.39 to 2.35), mean recovery by the UPRO method was 115% (vs. 63% for pyrogallol red and 83% pyrogallol red-SDS). Results positively correlated with A/G ratio for UPRO ($r = 0.69$; $P < 0.001$), pyrogallol red ($r = 0.48$; $P < 0.05$), but not pyrogallol red-SDS ($r = 0.191$; NS).

The difference between UPRO and pyrogallol red assays correlated with the A/G ratio ($r = 0.82$; $P < 0.001$). In light chain proteinuria ($n = 10$), no significant difference ($< 15\%$) was observed between techniques, whereas in glomerular selective proteinuria ($n = 10$), values were significantly higher with the UPRO assay (2.20 ± 1.61 vs. 1.43 ± 1.10 g/L; $P < 0.02$). Our results support the idea that screening for renal diseases can be performed with UPRO or pyrogallol red assays. However, since A/G ratios may vary with renal disease evolution, follow-up of patients with positive proteinuria should be performed using the same assay, preferably the pyrogallol red-SDS. *J. Clin. Lab. Anal.* 15:40–42, 2001. © 2001 Wiley-Liss, Inc.

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INTRODUCTION

Measurement of total urinary protein is used as a screening test in the biological assessment of disease conditions as varied as renal failure, preeclampsia, or multiple myeloma (1,2). Because of excellent analytical performances, high convenience, and low cost, dye-binding methods are among those most commonly used in laboratories. However, large variations in dye reactivity with different proteins exist, as demonstrated for example for the pyrogallol red with albumin and light chain of immunoglobulins (3). Johnson and Johnson recently developed a test (UPRO) based on urine protein reaction with the pyrocatechol violet-molybdate complex. According to the manufacturer, recovery data obtained with normal urine samples spiked with protein, are about 98% for albumin, but reach only 30% for light chains. Lynch et al. demonstrated a strong positive bias in measuring proteinuria with UPRO compared to the pyrogallol red method, but protein composition of their samples was not determined (4).

Thus, the influence of urine protein composition on UPRO assay in pathological samples remains largely unknown. This issue is, however, of major clinical importance because urinary protein composition strongly varies according to renal diseases: albumin in nephrotic syndrome, light chains in myeloma, low molecular proteins in tubular nephropathy.

In this study, we evaluated the influence of protein composition on total urinary protein determination by Pyrogallol red-molybdate methods both with and without sodium dodecyl sulfate (SDS), and by the UPRO assay. Mixtures of pure protein solutions, dilution of sera from myeloma patients, and urinary samples were used.

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MATERIALS AND METHODS

Total urinary protein concentration was determined by reaction with the pyrocatechol violet-molybdate complex (UPRO) on a Vitros 950 analyzer (Ortho Diagnostic System, Paris, France), and with Pyrogallol red with or without SDS automated on a Hitachi 747 apparatus (Roche Diagnostics, Grenoble, France). Pyrogallol red with SDS was from Merk-Clevenot (Nogent sur Marne, France) and Pyrogallol red without SDS was from Biomérieux (Marcy l'Etoile, France). Both Pyrogallol assays calibrators were pure human albumin solutions (1g/L), and UPRO was calibrated with aqueous solutions of bovine serum albumin (4). All tests are linear up to 2g/L (manufacturer's data).

We evaluated protein recovery with two experimental models with known protein composition (A/G ratio) and protein concentration (1g/L). Analyses were performed in duplicate and recovery (expressed in %) was calculated as the ratio between the observed and expected values of protein.

Mixtures (n = 8) of Pure Albumin and γ -globulins With Varying A/G Ratio

Human albumin (> 96% albumin) and γ -globulin (from Cohn fraction II, III purity > 99%) were from Sigma (Saint Quentin Fallavier, France). Stock solutions were prepared by gravimetry. Proteins were dissolved in NaCl (155 mmol/L). Final dilutions were made in distilled water.

Diluted Sera From Myeloma Patients (n = 26)

Total serum protein was assayed by the Biuret reagent adapted to a Hitachi 747 analyzer. Dilutions were made in distilled water. A/G ratio was determined by agarose gel electrophoresis with the "Hydragel" kit (Sebia, Issy les Moulineaux, France). The gels were stained by amidoschwartz at the concentration of 4g/L and scanned with the HR "Preference" system (Sebia). The A/G ratio was defined as the ratio between the area of albumin and globulins measured by densitometric integration.

Two groups of urinary samples were also selected according to their A/G ratio: light chain proteinuria (Bence Jones) (BJ) samples with an A/G ratio ≤ 0.1 (n = 10), and glomerular selective proteinuria (GSP) samples with an A/G ratio ≥ 9 (n = 10). A/G ratio was determined by agarose gel electrophoresis with the "Hydragel Proteinurie" kit (Sebia). Staining of gels was realized by acid violet at the concentration of 2 g/L and the gels scanned with the HR "Preference" system (Sebia, Issy les Moulineaux, France). The A/G ratio was defined as previously described.

RESULTS

For mixtures of pure albumin and γ -globulin (A/G ratio from 0 to 10), mean recoveries in the pyrogallol red, pyrogallol red SDS, and UPRO were 79% ($\pm 2.2\%$), 77% ($\pm 2.1\%$),

and 81.2% ($\pm 12.1\%$). In contrast to pyrogallol red methods, a significant positive linear relation was observed between A/G ratio and UPRO results ($r = 0.94$; $P < 0.001$) (Fig. 1).

For diluted myeloma sera (A/G ratio range, 0.39 to 2.35; mean, 1.05 ± 0.55), mean recovery by the UPRO method was 115% (range 81–164%) and correlated positively with the A/G ratio ($r = 0.69$; $P < 0.001$). Mean recovery with pyrogallol red SDS was 83% (range 66–114%) and did not correlate with the A/G ratio ($r = 0.191$). In the method with pyrogallol red without SDS, mean recovery was 63% (range 49–80%) and correlated with the A/G ratio ($r = 0.48$; $p < 0.05$) (Fig. 2). In this model, the difference between the UPRO and the two pyrogallol red assays (y) correlated with the A/G ratio (x): $y = 0.234x + 0.167$; $r = 0.82$; $P < 0.001$; $n = 52$; $S_{(y/x)} = 0.089$. For samples with A/G ratio < 1.0 (n = 22), no correlation could be observed between the UPRO and the two pyrogallol red assays protein values ($r = 0.087$; $P = 0.18$). For samples with A/G ratio > 1.0 (n = 30), the UPRO and the two pyrogallol red assays protein values were correlated as $y = 0.149x + 0.303$; $r = 0.755$; $P < 0.001$; $n = 30$; $S_{(y/x)} = 0.052$.

For BJ urine samples, no significant difference was observed between the UPRO and pyrogallol red protein values (2.02 ± 1.16 g/L vs. 2.28 ± 1.21 g/L, respectively; NS). With GSP urine samples, proteinuria values in the UPRO assay were significantly higher (+ 53%) than those obtained with the pyrogallol red assay (2.20 ± 1.61 g/L versus 1.43 ± 1.10 g/L, respectively; $P < 0.02$).

DISCUSSION

There is currently no recognized standard, and no universally accepted reference method for measurement of total

Fig. 1. Influence of the albumin/globulin ratio on total protein recovery in a mixture of pure human albumin and γ -globulins (at the final concentration of 1 g/L). Each point represents the mean of two experiments. $\circ$, pyrogallol red-SDS; $\bullet$, pyrogallol red; +, UPRO.

Recovery (%)

Fig. 2. Influence of the albumin/globulin ratio on total protein recovery in diluted sera from myeloma patients at the final concentration of 1 g/L. Each point represents the mean of two experiments. ○, pyrogallol red-SDS; ●, pyrogallol red; +, UPRO.

urinary protein. Any such method should, ideally, meet the criteria of excellent precision and sensitivity and be unaffected (or poorly affected) by protein composition, i.e., the A/G ratio. If analytical performances of existing methods are recognized (3,4), protein composition clearly influences their reactivities with different dyes. Use of different technologies for determination of total urinary protein within the same laboratory raises the important question of the routine monitoring of hospitalized patients. In the present study, we assessed the influence of the A/G ratio on UPRO test and pyrogallol red assays and protein recovery using two different approaches. A simple but rough model is the use of dilutions of pure stock protein solutions (containing only human albumin and γ -globulins). Second, dilutions of serum samples from multiple myeloma better reflect protein heterogeneity encountered in clinical urine samples: albumin, alpha-1, and alpha-2 globulins, light chains, and/or intact immunoglobulins (monoclonal and/or polyclonal) (2).

For the UPRO assay, total urinary protein was clearly influenced by the A/G ratio regardless of the experimental approaches selected: either the mixture of pure albumin and γ -globulins or diluted sera from myeloma patients. Protein recovery was lower at low A/G ratios than at high A/G ratios (Figs. 1 and 2). The pyrogallol red test, seemed less sensitive,

especially when SDS was added to the reagents, to A/G ratio than the UPRO test, meanwhile exhibiting an underestimation of total protein concentration. These results are in good agreement with those of Watanabe (5), who demonstrated that protein recovery with the pyrogallol red technique was undervaluated when the A/G ratio was low (about 80% with pure γ -globulins).

While UPRO and pyrogallol red underestimate globulins, this may not accurately reflect the extent to which these assays underestimate BJ proteinuria in clinical samples. Interestingly, both methods gave similar results for pure Bence Jones proteinuria (pyrogallol red + 13% vs. UPRO, NS). Because absolute differences between the two assays correlated with the A/G ratio ($r = 0.82$; $P < 0.05$), the largest discrepancies between the UPRO and pyrogallol red methods would be observed in urine samples containing a large majority of albumin (high A/G ratio), i.e., in selective glomerulonephritis. This is in agreement with our results obtained with GSP urine samples (53% higher with the UPRO assay). But assessment of low-molecular-weight proteinuria by the UPRO assay remains to be evaluated.

Our results support the idea that the renal disease screening of patients can be realized by both methods. The follow-up of patients with positive proteinuria should, however, be performed with a single assay since the A/G ratio may vary with renal disease evolution. We recommend that a SDS-pyrogallol red technique be preferred to the UPRO assay for urinary protein determination because it is less affected by the A/G ratio.

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