False-Negative Urine Protein Electrophoresis by Semi-automated Gel Electrophoresis, David F. Keren,* Ronald Gulbranson,1 and Stephen J. Ebrom2 (1 Warde Medical Laboratory, Ann Arbor, MI; 2 St. Mary’s Mercy Medical Center, Grand Rapids, MI; * address correspondence to this author at: 5025 Venture Dr., Ann Arbor, MI 48108; fax 734-665-0668, e-mail kerend@wardelab.com)

Urine is evaluated by electrophoretic methods both to gather information about the location and degree of damage within the nephron and to detect and quantify monoclonal free light chains (Bence Jones proteins). For many years, urine protein electrophoresis consisted of concentrating the urine (usually >50-fold) followed by manual application of the concentrated sample to a gel. Recently, semiautomated methods have become available that allow for a more efficient application of urine to the gels.

The Sebia Hydragel \(1\)-15/30 (Sebia, Inc.) method provides urine gel results with crisp separation of \(\beta\)-region bands as recommended by the guidelines for clinical and laboratory evaluation of patients with monoclonal gammopathies (1). However, in a recent urine sample, despite the presence of 2288 mg/24 h of total protein, the urine protein electrophoresis gel failed to demonstrate the proteinuria although the urine had been concentrated 50-fold (Fig. 1A). The technologist had observed sediment in the concentrated urine specimen. Because of the discrepancy between the total protein concentration and the appearance of the urine on electrophoresis, the electrophoresis on this sample was repeated on the next run. Before this run, the urine was centrifuged for 5 min in a fixed-head Sero-fuge (Clay Adams). Studies on urine samples have shown that this centrifugation has no effect on serum protein migration (data not shown). After centrifugation, the clear supernatant was placed in the applicator well. The second electrophoresis demonstrated a glomerular and tubular proteinuria with a suspicious broad \(\gamma\)-region band (Fig. 1B). The difference in the two patterns indicated that no sample had been applied to the gel during the first analysis.

The Sebia semiautomated agarose gel electrophoresis method uses plastic applicators (product no. 4199; Sebia) to apply the urine to the gel (Fig. 1C). Samples are pipetted into a plastic well, the bottom of which is a microporous membrane (Sebia) that leads to a straight edge. The liquid is drawn by wick effect into the microporous membrane and then to its edge. The applicator touches the edge of the microporous membrane to the gel, which places a small amount of the urine at the point of origin for the electrophoresis. The manufacturer’s instructions advise that urine with a high salt content may cause deformation of the gel. The instructions also note that use of old, improperly stored urine samples, in which enzymatic degradation of proteins might occur, should be avoided. During customer training, the company instructs

Fig. 1. Two Sebia \(\beta\)1,2 gels containing several concentrated urine samples (A and B; the second sample from the top in A is repeated after centrifugation as the second sample from the top in B), and close-up view of a portion of the application device (C).

(A), Sebia \(\beta\)1,2 gel containing several concentrated urine samples. The second sample is urine from the present case, and it appears to contain no protein (Sebia \(\beta\)1,2 gel stained with acid violet stain). (B), the sample in the second lane from the top is the repeat evaluation of the clear supernatant of the urine from the present case. Several protein bands are visible (Sebia \(\beta\)1,2 gel stained with acid violet stain). (C), close-up view of a portion of the plastic device used to apply urine to the agarose gel. Two of the 16 wells are shown. The bottom of the well contains the microporous membrane that passes behind the plastic front and ends in the edge for the application. The urine wicks along the microporous membrane to this edge, which is touched down to the agarose to apply the sample to the gel.
technologists to visually inspect the microporous membrane to ensure that the sample has wicked down the membrane properly. Lastly, the manufacturer recommends that the analysis be performed on urine concentrated to a total protein concentration of ~15–20 g/L. In the case of the sample described above, urinalysis demonstrated the presence of 443 leukocytes, 162 erythrocytes, and 3 epithelial cells per high-power field. The presence of debris associated with these cellular elements may account for the sediment observed by the technologist, possibly accounting for interference with the wicking of the urine onto the microporous membrane applicator.

By correlating the electrophoretic findings of urine with the total protein, we were able to avoid a false negative. Such comparisons have been recommended by Umbreit and Wiedemann (2). However, in more subtle cases, a small monoclonal free light chain could have been missed. Therefore, in addition to comparing all urine electrophoresis results with the total protein concentration, we recommend use of a clear supernatant when particulate matter is present in urine samples and examining the edge of the microporous membrane applicator to be certain that the urine has migrated to its edge.

References

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Extent of Aminoglycoside Interference in the Pyrogallol Red-Molybdate Protein Assay Depends on the Concentration of Sodium Oxalate in the Dye Reagent, Thomas Marshall* and Katherine M. Williams (Analytical Biochemistry Group, Sunderland Pharmacy School, The University of Sunderland, Sunderland SR1 3RG, UK; * author for correspondence: fax 44-191-515-3405, e-mail tom.marshall@sunderland.ac.uk)

The Pyrogallol Red-molybdate (PRM) reagent of Fujita et al. (1) has been modified by Watanabe et al. (2) for urinary protein determination. The assay has been widely adopted for this purpose, but interference from aminoglycoside antibiotics can produce falsely increased urinary protein values in acute-care patients, and the extent of interference varies with different PRM reagents (3, 4). Thus, the Dade Behring and Sigma reagents are susceptible to interference, whereas the Cobas Fara and Roche Integra 700 reagents are resistant (3, 4). The present study compares the PRM reagents of Fujita et al. (1) and Watanabe et al. (2) and demonstrates that the aminoglycoside interference varies with the concentration of sodium oxalate in the dye reagent.

Amikacin (cat. no. A1774), dihydrostreptomycin (cat. no. D7253), genetin (cat. no. G5013), gentamicin (cat. no. G1914), kanamycin (cat. no. K4000), neomycin (cat. no. N5285), paromomycin (cat. no. P9297), streptomycin (cat. no. S6501), and tobramycin (cat. no. T1783) were purchased from Sigma-Aldrich. Aqueous aminoglycoside solutions (10 g/L) were prepared gravimetrically and diluted to 1 g/L before assay. Pierce Prediluted Protein Assay Standard (1 g/L bovine serum albumin 23208) was purchased from Perbio Science UK Ltd. Urine control (AU2353) was purchased from Randox Laboratories Ltd. and reconstituted in deionized water or aqueous aminoglycoside (final concentration, 0.2 or 1.0 g/L).

The Fujita dye reagent was freshly prepared by mixing 300 mL of 0.2 mol/L HCl–0.2 mol/L sodium acetate buffer (pH 2.5) with 100 mL of 20 g/L gum arabic, 5 mL of 2.4 g/L disodium molybate, 67 mL of dye solution [60 mg of Pyrogallol Red (cat. no. P8759; Sigma Aldrich) in 100 mL of methanol], and 33 mL of methanol and adjusting the final volume to 1 L with deionized water (1). The reagent was modified to include 0.1, 0.5, 1.0, 2.0, or 4.0 mmol/L sodium oxalate by adding 0.4, 2.0, 4.0, 8.0, or 16.0 mL of 35 g/L sodium oxalate before adjusting the volume. The Watanabe dye reagent was freshly prepared by dissolving 5.9 g of succinic acid and 0.5 g of sodium benzoate in 900 mL of water and mixing with 40 mL of dye solution (60 mg of Pyrogallol Red in 100 mL of methanol). Four milliliters of 2.4 g/L disodium molybdate and 4 mL of 35 g/L sodium oxalate (final concentration, 1 mmol/L) were added; the mixture was adjusted to pH 2.5 with 0.5 mol/L hydrochloric acid and brought to a volume of 1 L with deionized water (2). The reagent was modified by omitting the sodium oxalate or varying its concentration between 0.1 and 4.0 mmol/L. For protein assay, 20 μL of aminoglycoside, urine control (with or without aminoglycoside), or protein calibrator was mixed with 1 mL of PRM reagent. After 30 min, the absorbance (600 nm) was measured with a Jenway 6100 spectrophotometer zeroed with a water/reagent blank.

The PRM reagent of Fujita et al. (1) was more sensitive to aminoglycoside interference than the reagent of Watanabe et al. (2) (Table 1). Modification of the Fujita reagent by adding sodium oxalate (0.1–4.0 mmol/L) progressively reduced the interference (Table 1). Thus, modified reagent containing 1.0 mmol/L sodium oxalate had a susceptibility to interference comparable to the Watanabe reagent, whereas reagent containing 2.0 or 4.0 mmol/L sodium oxalate was less susceptible to interference than the Watanabe reagent (Table 1). In contrast, modification of the Watanabe reagent by reducing the sodium oxalate concentration below the recommended 1.0 mmol/L (2) progressively increased the aminoglycoside interference (Table 1). Thus, modified reagent prepared without sodium oxalate gave high interference comparable to the Fujita reagent (Table 1). Conversely, modification of the Watanabe reagent by increasing the sodium oxalate concentration to 2.0 or 4.0 mmol/L progressively reduced the interference (Table 1).

The effect of sodium oxalate on aminoglycoside inter-