Sandy Hite, Carlos De La Barra, Lisa Tran, Denise Riggan, Scott Neynaber, Chris Eng, and Ron Williams at Gen-Probe.

References

Sensitive Method for Detection and Semiquantification of Bence Jones Protein by Cellulose Acetate Membrane Electrophoresis Using Colloidal Silver Staining, Kazuyuki Matsuda,1 Nobuo Hiratsuka,1 Takatoshi Koyama,1 Yuriko Kurihara,1 Osamu Hotta,2 Yoshihisa Itoh,3 and Kiyoko Shibata2 (1 Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8519, Japan; 2Department of Neurology, Sendai Shakkaihoken Hospital, Tsutsumimachi, 3-16-1, Aoba-ku, Sendai 981-8501, Japan; 3Department of Laboratory Medicine, Asahikawa Medical College, Miorigaoka-higashi 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan, *Author for correspondence: fax 81-3-5803-0166, e-mail k.shiba.mtec@tmd.ac.jp)

The monoclonal free light chain of immunoglobulin, Bence Jones protein (BJP), is associated with malignant mononuclear gammapathies, in particular with multiple
myeloma, lymphoproliferative diseases such as Waldenström macroglobulinemia and malignant lymphoma, amyloidosis associated with light chain, and light chain deposition disease (1,2). The detection of urinary BJP is useful for diagnosing and evaluating the prognosis for monoclonal gammopathies (3–7). BJP can be detected as a sharp band by urinary protein electrophoresis (UPE). UPE on cellulose acetate, cellulose nitrate, and agarose has been reported. More recently, sodium dodecyl sulfate-agarose gel electrophoresis and capillary electrophoresis of urinary proteins have been reported (8–13).

Electrophoresis on cellulose acetate membranes is carried out for serum protein fractions in many clinical laboratories, and it is a simple and easily reproducible technique. After UPE on cellulose acetate, membranes are stained with solutions containing Acid-violet 17 and Coomassie Brilliant Blue; however, extensive preconcentration of urine before electrophoresis generally is recommended because of the low concentrations of urinary proteins. This procedure for concentrating urine is time-consuming and has problems such as protein loss, aggregation, and degradation. Although highly sensitive staining methods using colloidal gold solution without preconcentration of urine have been reported (14–16), these methods require 2–3 h for protein staining.

We previously reported a rapid and highly sensitive colloidal silver staining solution suitable for cellulose acetate membranes (17). In this study, we further modified the staining method and developed a more rapid and sensitive colloidal silver staining method to detect small amounts of BJP that does not require preconcentration of urine. We used urine samples obtained from inpatients with multiple myeloma (n = 9), benign monoclonal gammopathy (n = 1), primary macroglobulinemia (n = 1), light chain deposition disease (n = 1), and primary amyloidosis (n = 5). Informed consent was obtained from all patients. The urinary total protein concentration was determined by the Pyrogallol red dye method (Wako Pure Industrial Chemical) on a Hitachi 7070 automated analyzer (Hitachi). Colloidal silver solution was prepared according to the following method. To a solution containing 12 mL of 2.5 mL/L Tween 20 and 15 mL of 28 mmol/L iron(II) sulfate heptahydrate, 5 mL of 280 mmol/L trisodium citrate dihydrate was added and mixed. One milliliter of 350 mmol/L silver nitrate was then added and vigorously shaken by hand to prevent premature floccu-

Fig. 1. Detection and identification of urinary BJP using our method and routine IFE (A) and semiquantification of urinary BJP (B).

(A), urine sample obtained from a patient with benign monoclonal gammopathy (urinary total protein, 57 mg/L; BJP, 5 mg/L). (Right), electrophoretic patterns obtained by our method with colloidal silver staining. (Left), routine IFE. UPE, electrophoretic pattern of urine proteins; G, anti-IgG; A, anti-IgA; M, anti-IgM; κ, anti-κ light chain; λ, anti-λ light chain. (B, left), electrophoretic image of albumin calibrators and urine sample. Lanes S1–S7, albumin calibrators S1 (2.5 mg/L), S2 (5.0 mg/L), S3 (10 mg/L), S4 (50 mg/L), S5 (100 mg/L), S6 (150 mg/L), and S7 (200 mg/L), respectively; lane U, urine sample. (B, right), representative calibration curve based on albumin calibrators (2.5–200 mg/L).
lution of silver particles. Finally, 2 mL of 150 mL/L acetate acid was added and mixed. This solution was prepared fresh before use. After electrophoresis at a constant current of 0.7 mA/cm per membrane for 25 min in veronal buffer (60 mmol/L, pH 8.6; ionic strength, 0.06), the proteins were fixed in 0.4 mol/L trichloroacetic acid–0.03 mol/L sulfosalicylic acid for 5 min, immersed in 10 mL/L acetic acid for 5 min, and stained with freshly prepared colloidal silver solution for 20 min with continuous shaking. Finally, the membranes were washed in distilled water for 10 min. For immunofixation (IFE), each track was overlayed with cellulose acetate strips impregnated in antisera specific against IgG, IgA, IgM, and free and ground noise, urine samples from 74 patients without BJP were analyzed by UPE combined with IFE using our colloidal silver staining solution. and 32 healthy volunteers were analyzed by UPE combined with IFE using our colloidal silver staining solution.

As shown in Fig. 1A, we could accurately detect and identify BJP from unconcentrated urine that had been obtained from a patient with benign monoclonal gammopathy (total urinary protein, 57 mg/L; BJP, 5 mg/L). On the other hand, routine IFE detected a small amount of BJP that was not apparent after UPE. Patient profiles and data are summarized in Table 1. We could detect and identify BJP for 32 healthy volunteers were analyzed by UPE combined with IFE using our colloidal silver staining solution. without preconcentration of urine samples, we generated a calibration curve using the areas for densitometric images of albumin calibrators, and the BJp concentrations obtained using the present method (y) and those obtained using the immunonephelometric assay (x). The linear regression equations for BJp was: 

$$y = 0.777x + 57.661 (r = 0.999)$$

With our highly sensitive silver staining method, even 5 mg/L BJp could be detected by staining for only 20 min. In addition, the colloidal silver staining solution we developed can also be used for IFE to determine the type of BJp and to identify other proteins. Even when IFE is performed, the entire procedure is completed in only 1.5 h. The sensitivity of our method has been shown to be higher than that of routine IFE with ~100-fold concentrated urine. In preliminary studies to ascertain background noise, urine samples from 74 patients without BJp and 32 healthy volunteers were analyzed by UPE combined with IFE using our colloidal silver staining solution.
We did not detect BJP and had no false-positive results (data not shown). Given the occurrence of false positives in cases such as hepatitis where oligoclonal banding is common, interpretation of the result together with other clinical data is important.

Increased BJP indicates a poor prognosis and aggravation of monoclonal gammopathies. The quantification of urinary BJP is important for observing the course and evaluating treatment effects. In patients, the urinary total protein concentration varies. Because low urinary total protein concentrations cannot be determined precisely, the BJP concentration cannot be calculated by multiplying the amount of total protein by the percentage of the BJP band. When we used χ or λ solutions as calibrators for semiquantification of BJP, their electrophoretic patterns were smeared. In this study, therefore, we used albumin as a calibrator, for which the electrophoretic pattern was a single sharp band. The calibration curve based on albumin calibrators has been shown to be acceptably linear. More accurate quantification is possible in overflow-type urine samples, which show a high correlation between the values obtained by our semiquantitative method and that obtained by immunonephelometry. However, for electrophoretic images of urine samples containing high amounts of protein, a decrease in the quantification accuracy may also be taken into consideration. In the recent study by Levinson (18), BJP concentrations were expressed as semiquantitative values based on data obtained by UPE and IFE. If the results obtained by our method are also considered to be semiquantitative rather than quantitative, our method may be useful for observing and evaluating the clinical course of monoclonal gammapathies.

This study was supported in part by a research grant from the Kurozumi Medical Foundation. We thank Yoji Hirabayashi of SRL Inc. for performing the immunonephelometry assay and Naoko Yusa (Department of Nephrology, Sendai Shakahoken Hospital) for collection of urine samples and clinical information.

References

Stability of Busulfan in Frozen Plasma and Whole Blood Samples, Poonekuzhal Balasubramanian, Alok Sriwastava, and Mammen Chandy (Department of Hematology, Christian Medical College Hospital, Vellore, India; *author for correspondence: fax 91-416-232035, e-mail bala@hemato.cmc.ernet.in)

Busulfan is widely used as a component of myeloablative conditioning therapy for bone marrow transplantation (1–5). Busulfan is most often used at a fixed total dose of 16 mg/kg. Wide interindividual variation in the bioavailability of busulfan has been recognized. Pharmacokinetic analyses to achieve target plasma concentrations and dose adjustments are increasingly being used to improve the outcome of bone marrow transplantation (6–8). Studies on busulfan kinetics have demonstrated that busulfan concentrations in stored plasma samples are stable for up to 3 months at −20 °C and that blood samples for busulfan analysis should be centrifuged within 3 h of collection and plasma frozen if not analyzed immediately (9–11). The stability of busulfan in blood and plasma samples stored for longer time periods and at lower temperatures has not been reported. Because pharmacokinetic analysis of busulfan requires collection of multiple samples at frequent intervals, such data will be useful in planning transport and analysis of blood and plasma samples.

This study was undertaken to determine the stability of busulfan in whole blood samples stored up to 24 h at 4 °C and in plasma samples stored up to 2 years at −80 °C. 

Busulfan analysis was performed on plasma samples by HPLC as described previously (12). Briefly, busulfan in plasma was extracted with toluene and derivatized with 1 mol/L tetrafluoroethanol under alkaline conditions for 2 h at 70 °C. The tetrafluoroethanol derivative of busulfan thus formed was reextracted with 1 mol/L