BJP static concentrators from Pro-Chem: Tools to concentrate Bence Jones Protein in urine

Dr Mike Brownleader

Pro-Chem Inc. 521 Great Road, Littleton, MA 01460, USA (WWW: www.protein-chem.com)

Background

Bence Jones proteins (BJP; M_r 22,000) were described by Dr Henry Bence Jones and others in 1845 and are monoclonal free light chains (Kappa, κ or lambda, λ) produced after a proliferation of a single clone of plasma cells. The presence of Bence-Jones Proteinurea in urine is a valuable prognostic indicator for the presence of multiple myeloma (malignant proliferation of white blood cells called plasma cells). Multiple myeloma consists of 15-20 % of all monoclonal gammopathies. Other clinical indicators are increased levels of myeloma cells in bone marrow and the destruction of bone either as osteolytic lesions or as extreme osteoporosis (Ward et al, 1999). If only two of these features are present, a confident diagnosis can be made.

Average incidence rates of 3-5 per 100,000 population are common in Europe and USA and reports show that mortality rates from multiple myeloma have been increasing dramatically over the past three decades. In African-Americans, the rate of incidence of multiple myeloma increases to nearly 10 per 100,000 population. It remains one of the 10 leading causes of cancer death. Each year, according to the American Cancer Society, 13-15,000 people in the United States learn that they have multiple myeloma. These people will undergo regular urine tests during follow-up examinations to monitor treatment methods. The patient's serum and urine will be monitored every 3-6 months for the rest of their lives. If the chemotherapy is stopped, the patient will undergo benign maintenance therapy and the clinician will monitor the urine/serum for BJP recurrence.

Urinary Bence Jones protein

The level of Bence Jones protein in urine and serum depends upon the progression of the disease. BJP is found in urine as a result of overflow proteinuria and tubular damage caused by the toxic effects of high levels of BJP. In the early stages of the disease only low amounts of BJP are produced because BJP is easily reabsorbed by the tubules of a healthy kidney. As a result, BJP does not appear in urine. As the disease evolves, overflow proteinuria occurs as serum concentrations of BJP exceed the reabsorptive capacity of the tubules. In this case, BJP can be detected in both serum and urine. When the disease is at an advanced stage, large amounts of BJP are excreted into the urine even though serum concentrations of BJP may not be significant.

Since the proteins suspended in urine are often dilute, it is usually necessary to concentrate them before any analyses can be performed. It is estimated that 50 % of patients have monoclonal protein in the serum and urine, 25 % of patients have BJP in urine only and 25 % of patients have free monoclonal light chain in serum only. During chemotherapy, a patient with monoclonal proteins in serum may lose this protein marker and only produce BJP in urine. This is one reason why clinicians look for BJP in serum and urine. Bence Jones protein usually appears in levels that are below the detection limits of many traditional techniques such as cellulose acetate electrophoresis and capillary zone electrophoresis. A 10-100 fold concentration step is usually required before urine protein electrophoresis and subsequent protein staining with Amido Black or modified Coomassie Brilliant Blue prior to densitometry of the electrophoretic strip. A positive result is shown by identification of the characteristic protein bands in the gamma region of the gel. This is a lengthy process that requires skilled personnel as BJP does not always migrate to the same relative location in the gel.

Protein reference labs strive to measure 10 mg/l BJP in urine (Beetham, 2000; Graziani et al, 2003). It is estimated that 10 mg/l BJP equates to approximately 100,000 tumour cells which is a level indicative of minimal residual disease. A survey conducted by UK National External Quality Assessment Scheme (NEQAS) showed that 35 % of laboratories could not measure BJP at 60 mg/l (Ward and White, 1998). For diagnosis, concentrations of BJP > 100 mg/l are likely to indicate B-cell malignancy. Currently, many clinical haematologists are recommending more aggressive chemotherapies and so the ability to intensify BJP bands and monitor low levels of BJP is critical.

Static ultrafiltration concentrators

The static concentrator, first introduced in UK in 1970s, concentrates urine through the absorption of water by absorbent pads located on the underside of a 7.5 kDa-15 kDa molecular weight cut off ultrafiltration membrane. The molecular weight cut off is a measure of selectivity of an ultrafiltration membrane. It is defined as the point at which 90% rejection occurs for reference polymers of a defined and tight molecular weight range (Cardew and Le, 1998). Membranes are also characterized as having sharp or diffuse cut-offs depending upon the distribution of the size of the pores in the membrane. However, the MWCO of the membrane is dependent on the operating filtration conditions. Bence Jones protein will be retained on the membrane surface in the front compartment of the BJP unit because it has a 7.5 kDa MWCO membrane, a molecular weight cut-off far from the molecular weight of Bence Jones protein itself.

BJP clinical concentrators

BJP clinical concentrators were designed to optimize concentration and recovery of proteins for identification of monoclonal gammopathies by urine protein electrophoresis or immunofixation electrophoresis. Unlike other competitive products, these static concentrators were designed specifically to meet the demands of a busy protein reference laboratory concentrating urinary BJP. Pro-Chem's BJP concentrators are offered in 5, 10 and 20 ml benchtop units (Fig. 1). The 5 and 10ml formats are available either as single units or 8-test self-standing units (Fig. 2). Protein loss through the membrane is minimized by utilizing a low-binding proprietary-treated polyethersulfone membrane with a M_r 7,500 molecular weight cut off (MWCO). This is the ideal pore size to retain the Bence Jones proteins. The innovative and unique BJP format utilizes individual cells and membranes which prevent cross-contamination of samples.

BJP offer considerable time savings compared to similar static and pressure-driven products in the marketplace. These units enable multiple urine samples to be concentrated in parallel without high shear forces associated with a centrifuge. A 100-fold concentration of urine can typically be achieved in 30-40 min depending on the sample starting volume and protein concentration. Speed of filtration is affected by several parameters such as temperature, pH and protein concentration. Filtration speed will increase proportionately with ambient temperature. Concentration rates may decrease at lower temperatures. An acidic sample with a pH less than 5.0 will take longer to concentrate than a neutral pH sample. In this instance, pH can be adjusted to the physiological pH for faster filtration times. In addition, urine samples with high initial protein concentrations will concentrate slower than dilute samples. The vast majority of urine samples have initial protein concentrations less than 500 mg/dl. As a rule of thumb, urine samples with protein concentrations exceeding 2000 mg/dl will result in significantly slower filtration speeds.

Modus operandi of the BJP concentrator:

The urine sample is pipetted through an aperture at the top of the device (Fig. 3). The device can be left unattended until the desired concentration is achieved. BJP concentrators will prevent samples from concentrating to dryness due to an impermeable 50 μl deadstop

pocket. The concentration can be monitored using easy-to-read printed graduation marks. Once the desired concentrated volume is achieved, the concentrate is recovered using a plastic Pasteur pipette and is ready for further analysis.

Ease of use of the BJP concentrator:

With many other static concentrators, care is required to prevent the glass Pasteur pipette from breaking during loading and recovery of the final concentrate. Often the glass pipette is the last remaining raison d'etre for a laboratory to have a sharps disposal bin. The large opening of the BJP concentrator accommodates a plastic pipette, eliminating the need for glass pipettes and syringes and therefore in the majority of cases, a sharps disposal bin.

Some static concentrator products can only accommodate 5 ml samples. The BJP product range extends the initial sample volumes to 20 ml. In addition, other static concentrators require that sodium azide is added to the membrane to prevent microbial growth. Addition of 5 ml sodium azide would limit the solvent absorption capacity of the pads. The individual BJP units are disposable so microbial contamination is not possible. The 8-test BJP units have individual membrane cells and absorbents so that all unused cells are unaffected by tests performed in adjacent compartments. Therefore, BJP units do not require sodium azide treatment and no cross-contamination occurs.

Concentration rates and protein recoveries of the BJP unit:

A BJP 5 unit filled with 5 ml urine typically takes about 30-40 min for a 100 fold concentration. Recoveries of at least 95-98 % BJP are typical (Fig. 4). An 8-test competitive product averaged 90 min for a 100 fold concentration. The BJP 20 static concentrator performs a 100 fold concentration of 10 ml urine in 50 min. The hands-off operation allows the operator time to continue with other analyses in a busy laboratory environment while the device is left unattended to concentrate the urine samples. The impermeable dead stop pocket prevents the urine sample from concentrating to dryness.

Adsorption of proteins can occur whenever any solid surface is in contact with a protein solution. To minimize protein adsorption of dilute samples to the membrane surface, the 7.5 kDa MWCO membrane in the BJP static concentrator is pre-treated using a proprietary method to ensure full compatibility with body fluids and very low protein binding characteristics. Consequently, recoveries in excess of 95 % BJP are common with BJP units.

BJP

BJP concentrators offer a fast, gentle and convenient method to concentrate multiple clinical samples for analyses by electrophoresis and immunofixation. No pressure lines, vacuum pumps or centrifuges are required. The procedure is simple and straightforward. The operator loads the sample into the bench-top concentrator, waits for the sample to concentrate and recovers the protein concentrate with confidence.

References:

1. Ward A.M., Riches P.G., Fifield R. and Smith A.M. SAS Protein Reference Units: Handbook of Clinical Immunochemistry, 6th Edition, 1999, PRU Publications, Sheffield, UK NEQAS for Immunology and Immunochemistry.

2. Beetham R. (2000) Detection of Bence-Jones protein in practice. Ann. Clin. Biochem. *37*, 563-570.

3. Graziani M., Merlini G and Petrini C. (2003) Guidelines for the analysis of Bence Jones Protein. Clin. Chem. Lab. Med. *41(3)*, 338-346.

4. Ward A. M. and White P. Summary for UK NEQAS monoclonal protein distribution 986. UK NEQAS for monoclonal proteins. 1998, Sheffield, UK NEQAS for Immunology and Immunochemistry.

5. Cardew P.T. and Le M.S. Membrane Processes: A Technology Guide, 1998, The Royal Society of Chemistry, Cambridge, UK.

Figure Legends:

Fig. 1 showing the BJP 5, BJP 10 and BJP 20 individual static concentrators.
Fig. 2 showing the specifications of the 8-test BJP 5/40 and BJP 10/40 concentrators.
Fig. 3 highlighting the operation of the BJP 10/40 unit.
Fig. 4 showing an electrophoretogram of two BJP query urine samples pre-concentrated 50 fold using BJP concentrators.

Fig. 1

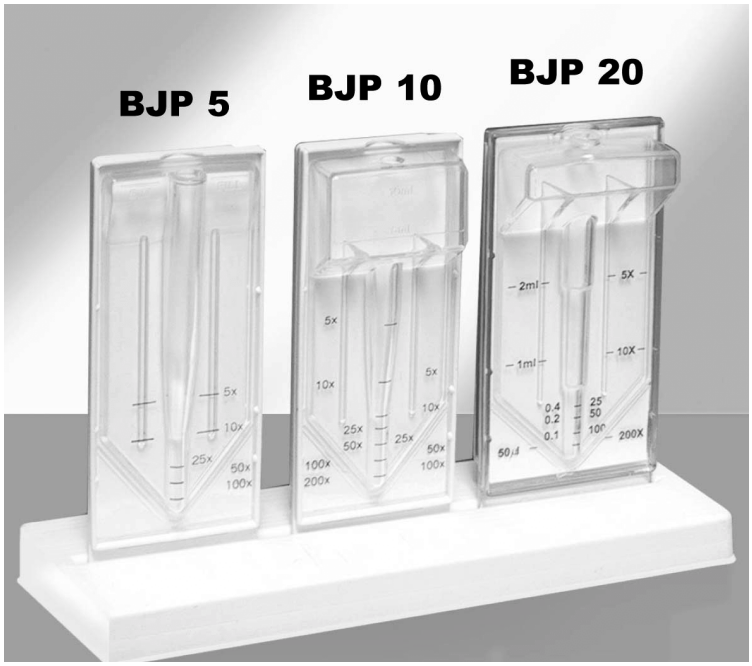


Fig. 2

Membrane Material	Polyethersulphone
Available MWCOs	7.5 kDa
Maximum sample volume with a reservoir	20 ml
Volume range (BJP 5)	0.5-5 ml
Volume range (BJP 10)	1-10 ml
Volume range (BJP 20)	2-20 ml
Max concentration factors (BJP 10 & 20)	200 x
Dead stop volume	50 µl
Effective membrane area	23 cm^2
Storage	Room temperature
Membrane wetting agent	Shipped dry in glycerine and sodium azide

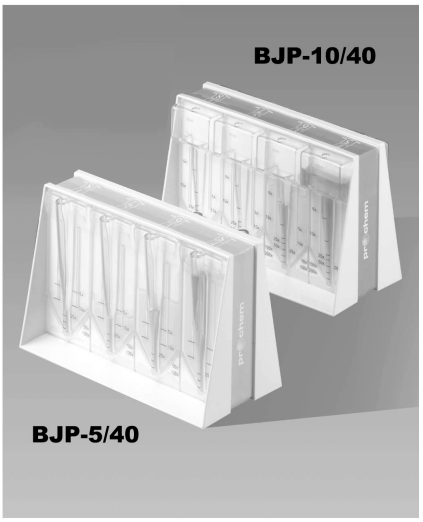


Fig. 3

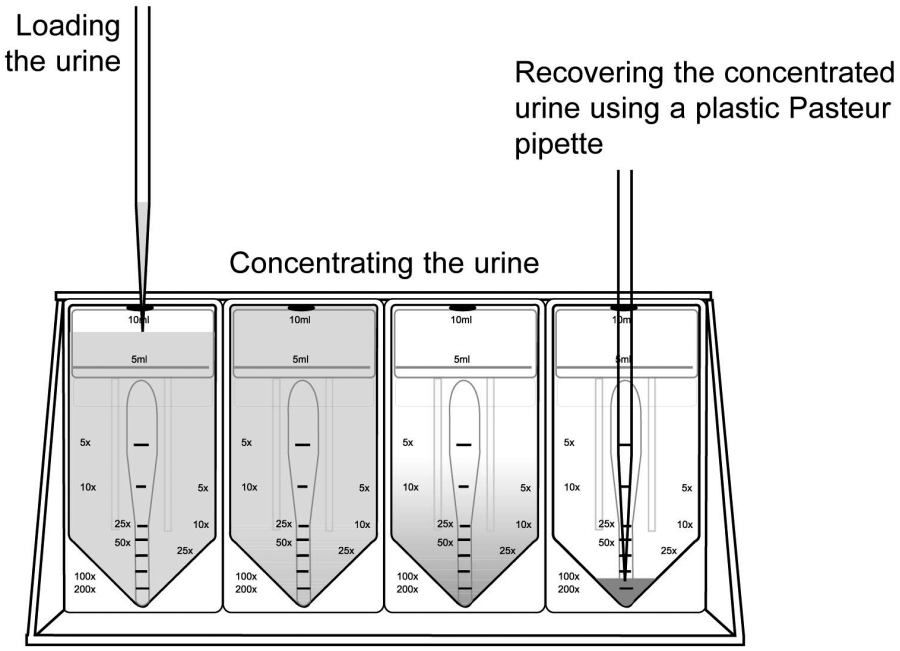
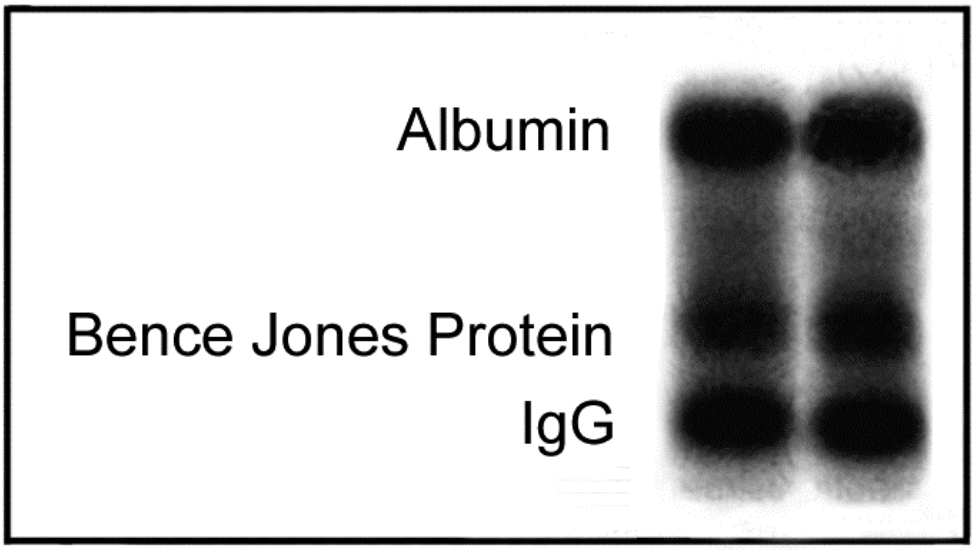


Fig. 4


Albumin
Bence Jones Protein
IgG