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Urine Protein Concentration with Vivaspin® Concentrators

Application Note

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turning science into solutions

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Background

Measurement of proteins in urine is important for the diagnosis and monitoring of a variety of diseases and disorders. Normally proteins larger than 100,000 daltons (100 kDa) such as immunoglobulins are retained in blood and much smaller molecules (<10 kDa) pass freely into the urine. Intermediate sized molecules such as albumin (~69 kDa) and free light chains (FLC) (~25 kDa) will pass into urine to varying degrees and then usually be reabsorbed by the nephron tubular system. However proteins can be excreted in urine as the result of several conditions. Proteinuria (excess protein in urine) is associated with glomerular and tubular diseases of the nephron as well as plasma cell disorders that cause elevated protein concentrations in the blood (overflow proteinuria). These plasma cell diseases include multiple myeloma (MM) and light chain amyloidosis (AL) and are diagnosed by the presence of monoclonal FLC, also known as Bence Jones protein (1).

The International Myeloma Working Group recommends that, after diagnosis of a plasma cell disorder is made, patients should be monitored by urine protein electrophoresis (UPE) and immunofixation electrophoresis (IFE). Additionally, initial screening for AL should be done with urine samples as well as serum (2). IFE uses antisera to identify the monoclonal protein (M-protein) as an immunoglobulin (IgG, IgA, IgM, IgD or IgE) or as a FLC. The FLC can be present as 25 kDa monomers (κ or K) or as 50 kDa dimers (λ or L). Most urine IFE samples are run with antisera for IgG, IgA, IgM, κ and λ since these represent over 90% of the M-protein isotypes (3).

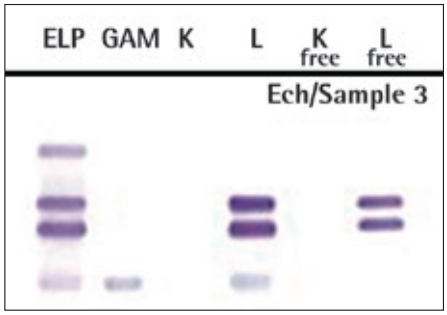

Figure 1: IFE of Concentrated (50×) Urine Sample
Urine sample IFE showing the presence of M-protein. Antisera selected for IgG/IgA/IgM (grouped as GAM) as well as K & L and free K & L chains. Sample shows two free L chains (dark bands in middle) and one L chain bound to a heavy chain (light band on bottom). (Photo provided by Sebia USA)

Many investigators report that 24 hour urine collection samples need to be adequately concentrated prior to UPE and IFE (4)(5)(6)(7). Densitometer scans of the UPE are then used to quantitate the amount of M-protein in the urine sample (4)(7). The required degree of concentration can vary according to the electrophoretic gel and the amount of protein in the sample. While many labs concentrate 50 – 100×, excessive concentration should be avoided since it can overload the gel (5). On the other hand, insufficient concentration can lead to not diagnosing some cases with M-proteins (8). The concentration factor (CF) is calculated by dividing the starting sample volume by the final volume.

Aside from UPE and IFE, capillary electrophoresis (CE) systems are also available and provide rapid, automated separations. The electropherogram from a CE system is similar to a densitometry scan. M-protein identification can be performed with CE using antisera and is referred to "immunotyping" by Sebia or "immunodisplacement" by Helena Labs.

Concentrators
Urine concentrators utilize ultrafiltration (UF) membranes which can retain proteins on the basis of their rated molecular weight cutoff (MWCO). While the proteins are filtered by the membrane, water, salts and other small molecules pass through thereby reducing the sample volume and concentrating the retained proteins. Water can be filtered through the UF membrane using centrifugal force or absorbent material behind the filter. In order to maximize recovery of M-proteins, the concentrator should have a membrane with a MWCO of 10 kDa or less.

Vivaspin® centrifugal concentrators are designed to be used with swinging bucket or fixed angle rotors. They use a patented (US 5,647,990) vertical membrane design with thin channel support to provide high speed filtration. For urine concentration, the 10 kDa MWCO is recommended for optimal recovery. The Vivaspin® have clearly marked volumes and dead stop compartments to prevent samples from concentrating to dryness. Vivaspin® are available for a variety of sample volumes ranging from 0.5 ml to 20 ml. Urine samples of 4 ml may be concentrated 50× in about 15 – 20 minutes depending on the initial total protein.

Most labs use Vivaspin® devices in the 4 – 6 ml range, for UPE and IFE while others, such as the Mayo Clinic, report a preference for the larger Vivaspin® 20 (6). Vivaspin® can yield high concentration factors (up to 200×).

Procedures
Samples for UPE or IFE are typically collected from 24 hour urine patient specimens. First the initial total protein (TP) should be measured using colorimetric dye binding or a similar method. Then the urine should be treated to remove any sediment which could interfere with the electrophoresis results (9). Such sediment can also slow down filtration rates during concentration and even totally obstruct the membrane. The sample can be clarified by use of a 10 – 20 µm disposable filter or by centrifuging the sample for about 5 minutes at 1000 – 2000 g.

As mentioned previously, the sample must be concentrated enough to provide visible bands after UPE and IFE yet not so much that the gel is overloaded. Laboratories will normally use the initial TP to determine the desired CF. The CF calculation is dependent on the minimum TP recommended for the gel being used. Most samples should be concentrated to at least 2-3 G/dL for UPE and slightly less for IFE but these numbers should be confirmed with the gel supplier. Since some labs require IFE volumes of up to 100 µl of concentrated urine (instead of about 20 µl for UPE), this final volume must be considered when calculating the desired CF.

After determining the target CF, most labs will first fill the sample reservoir to its rated capacity and stop the concentration process at the appropriate final volume. With a Vivaspin®, the centrifugation time is adjusted to yield the correct final volume but this can be a trial and error process. If a sample is concentrated too much, filtrate or purified water | buffer can be added back to reconstitute the sample to the desired volume. Other labs will reduce the starting volume in order to reduce the final CF. This method has been used for Vivaspin® and has the added benefit of reducing the centrifugation time since less sample has to be filtered.

Laboratories will usually generate a chart showing the target CF as a function of the starting TP concentration. Examples of charts are shown in Tables 1 and 2. The values shown in these tables depend on: (1) the type of concentrator, (2) choice of UPE or IFE, (3) the final desired TP for electrophoresis, and (4) the choice of constant or variable sample volume. Note that the CF values are only suggestions and increased concentration may be needed to detect faint M-protein bands in some cases. Following concentration, M-protein peaks found on the gel should be scanned and fractionated on a densitometer. Then the amount of M-protein in the 24 hour urine sample may be calculated by multiplying the amount of that fraction in the electropherogram by the starting urinary TP concentration (4)(7).

Initial TP Conc. (mg/dL)	Sample Volume (mL)	Conc. Volume (µL)	Conc. Factor	Final TP Conc. (G/dL)
< 25	8	100	80	< 2.0
25 – 50	4	100	40	1.0 – 2.0
51 – 100	2	100	20	1.0 – 2.0
101 – 250	1	100	10	1.0 – 2.5
> 250	0.4	100	4	> 1.0

Table 1: Urine Concentration Chart
Values for IFE using a Vivaspin® 4 with Variable Sample Volume and desired Final TP of 1.0 G/dL

Note
Two Vivaspin® 4 devices are used to provide enough sample for IFE.
Need 8 mL total sample concentrated to 50 µL in each Vivaspin®.

Starting TP Conc (mg/dL)	Sample Volume (mL)	Conc. Volume (µL)	Conc. Factor	Final TP Conc. (G/dL)
< 17	6	30	200	< 3.4
17 – 40	6	50	120	2.0 – 4.8
41 – 70	6	100	60	2.5 – 4.2
71 – 170	6	200	30	2.1 – 5.1
171 – 340	6	500	12	2.1 – 4.1
> 340	6	1000	6	> 2.0

Table 2: Values for UPE using a Vivaspin® 6 with Constant Sample Volume and desired Final TP of 2.0 G/dL

Capillary electrophoresis systems require urine samples to be prepared by ultrafiltration devices prior to analysis. Samples are first diluted with water and concentrated to remove salts. Then buffer is added and samples are centrifuged again to exchange the buffer. Sebia and Helena Labs both recommend the use of Vivaspin® 20 devices to prepare urine samples for their capillary systems (10)(11).

CAP Validation
Concentration procedures should be validated on a regular basis to comply with quality inspections conducted by the College of American Pathologists (CAP) (USA laboratories only). One popular method involves measuring TP recovery after concentrating urine samples according these steps:
(1) Prepare the urine as discussed previously and determine the initial TP (TP1).
(2) Fill the concentrator with the sample volume (V1) and perform the concentration.
(3) Measure the final volume (V2) accurately and then measure the final TP (TP2).
(4) Calculate the CF according to the equation CF = V1 | V2.
(5) Calculate the recovery (R) where R = 1000 × TP2 | (CF × TP1). The 1000 factor is to convert TP2 from G/dL to mg/dL. Multiply by 100 for %.

The sample results can be entered into a spreadsheet to calculate the average TP recovery (see example in Table 3). Labs should define their own quality criteria but 70 – 80% is usually acceptable.

Sample Number	TP1 – Starting Conc. (mg/dL)	V1 – Sample Volume (mL)	V2 – Conc. Volume (µL)	CF	TP2 – Final Conc. (G/dL)	Recovery R=1000 × TP2 | (CF × TP1)
1	30	4	50	80	2.0	83%
2	120	4	200	20	2.2	92%
3	18	4	20	200	2.9	81%
4	60	4	100	40	2.1	88%
Average						**86%**

Table 3: Urine Concentration CAP Validation Chart
Values for TP readings using Vivaspin® 4 devices with various patient samples.

Note that using TP is not completely accurate as a method to check recovery of M-proteins. TP values can include small proteins and polypeptides that are not clinically significant when diagnosing M-proteins. These small molecules can pass through the membrane and not be concentrated so they reduce the TP recovery %. Samples with higher TP values usually show higher recoveries since the small molecules represent a lesser percentage of the total.

Another method for validation is to perform a series of concentration tests on split samples. For example, the urine could be split into 5 samples of 5 ml each. Four of these could be concentrated to the following CF values: (1) 10×, (2) 25×, (3) 50× and (4) 100×. A UPE would be performed for each of these along with the unconcentrated (neat) sample. The bands of the UPE should become darker as the CF increases. Note that this is not a quantitative test but is used by some labs (see Figure 2).

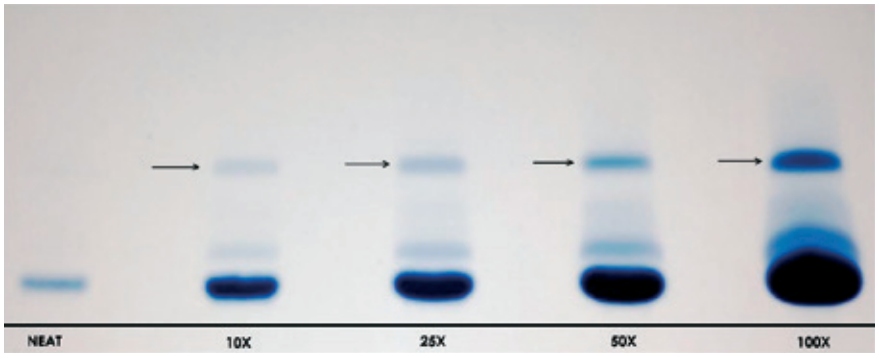

Figure 2: CAP Validation by Serial Concentration of Urine Sample

Patterns for UPE for a single urine sample with starting TP of 30 – 100 mg/dL (measured by Multistix 10). Albumin bands show on bottom & monoclonal FLC (Bence Jones protein) show on top (see arrows). Sample is split and concentrated to increasing CF as shown on bottom. Note that the Neat sample does not show a visible FLC band.

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* For Investigational Use Only: The performance characteristics of this product have not been established.

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Specifications subject to change without notice. Printed in the EU on paper bleached without chlorine. W
Publication No.: SL-4069-e150401 · Order No.: 85037-550-33 · Ver. 04 | 2015