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Urine Protein Concentration with Vivaspin®

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Abstract

Measurement of proteins in urine is important for the diagnosis and monitoring of a variety of diseases and disorders. Normally proteins larger than 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 re-absorbed by the nephron tubular system. However, these proteins can also be excreted in urine as the result of several conditions. To enable accurate and early diagnosis, it is often necessary to concentrate the protein content of urine samples prior to analysis. Vivaspin® centrifugal devices are ideal for this purpose, combining fast ultrafiltration with high concentration factors and recoveries, to reach the sensitivity required for accurate detection in subsequent electrophoretic techniques.

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Introduction

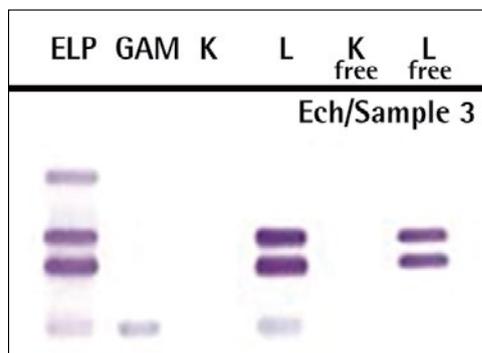
Proteinuria (excess protein in urine) is associated with glomerular and tubular diseases of the nephron as well as plasma cell disorders that cause elevated protein concentrations in the blood (overflow proteinuria). These plasma cell diseases include multiple myeloma (MM) and light chain amyloidosis (AL) and are diagnosed by the presence of monoclonal free light chains (FLC), also known as Bence Jones protein¹.

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 serum². 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 isotypes³.

Many investigators report that 24 hour urine collection samples need to be adequately concentrated prior to UPE and IFE^{4,5,6,7}. Figure 1 shows an example gel image, following urine sample IFE. Densitometer scans of a UPE are 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 \times , excessive concentration should be avoided since it can overload the gel⁵. On the other hand, insufficient concentration can lead to not diagnosing some cases with M-proteins⁸. 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 as “immunotyping” by Sebia or “immunodisplacement” by Helena Labs.

Figure 1: IFE of Concentrated (50 \times) 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 light chains (dark bands in middle) and one L chain bound to a heavy chain (light band on bottom). (Photo provided by Sebia USA)



Concentrators

Urine concentrators utilize ultrafiltration (UF) membranes which can retain proteins on the basis of their rated molecular weight cut-off (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 vertical membrane design with thin channel support to provide high speed filtration. For urine concentration, the 10 kDa MWCO is recommended for optimal recovery. Vivaspin[®] devices have clearly marked graduations for volume estimation, and integral dead-stop compartments to prevent samples from concentrating to dryness. Vivaspin[®] are available for a variety of sample volumes ranging from 0.5 to 20 mL. Urine samples of 4 mL may be concentrated 50 \times 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[®] 20⁵. Vivaspin[®] can yield high concentration factors of up to 200-fold.

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 results⁹. 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 μ m disposable filter or by centrifuging the sample for about 5 minutes at 1,000–2,000 *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 μ L of concentrated urine (instead of about 20 μ L 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 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}.



CAP Validation

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

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

Note: Two Vivaspin® 4 devices – each filled with 4 mL of sample and concentrated to 50 µL – are used to provide enough sample for IFE.

Table 2:

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

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	1,000	6	> 2.0

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}.

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 to 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:

$$R (\%) = \left\{ \frac{1,000 \times TP2}{CF \times TP1} \right\} \times 100$$

The 1,000 factor is to convert TP2 from g/dL to mg/dL.

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.

Table 3: Urine Concentration CAP Validation Chart

Values for TP readings using Vivaspin® 4 devices with various patient samples.

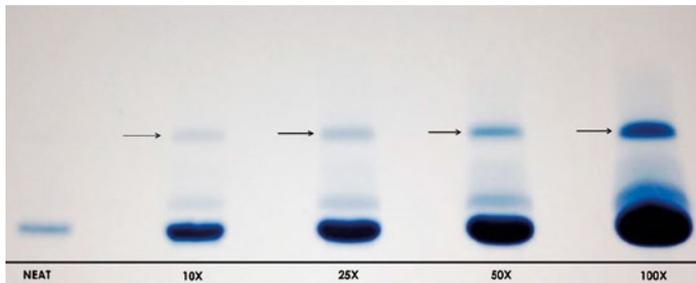
Sample Number	TP1 – Starting Conc. (mg/dL)	V1 – Sample Volume (mL)	V2 – Conc. Volume (µL)	CF	TP2 – Final Conc. (g/dL)	Recovery R = $\frac{1,000 \times TP2}{CF \times TP1}$
1	30	4	50	80	2.0	83%
2	120	4	200	20	2.2	92%
3	18	4	20	200	2.9	81%
4	60	4	100	40	2.1	88%
Average						86%

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.

References

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.

Conclusions

Vivaspin® centrifugal concentrators are important tools for the preparation of urine samples in clinical testing laboratories prior to *in vitro* diagnostics (IVD). Fast processing times coupled with high concentration factors and high recoveries of disease markers ensures increased sensitivity when performing subsequent analysis and diagnostics by IFE, UPE and CE.

Notes

Sartorius ultrafiltration products for IVD use are available only in selected regions, according to local regulations for IVD device registration. Please speak with your local Sartorius contact for the latest list of devices and countries in which they are available to purchase.

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

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