Chapter 2

A Primer on Clinical Applications and Assays Using Urine: Focus on Analysis of Plasma Cell Dyscrasias Using Automated Electrophoresis and Immunofixation

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Abstract

Urine is a noninvasive sample that is ideal for screening, because it is easy to collect, cost-effective, and can provide a wealth of information on a patient's health status. We provide a brief discussion on the anatomy and physiology of the kidney, a concise overview on B and T cells as key mediators of the immune system, and then delve into the various B-cell neoplasms. This discussion details Waldenstrom's macroglobulinemia, plasmacytoma, heavy chain disease, amyloidosis, and multiple myeloma. Of primary clinical importance from a technical perspective, two commonly applied techniques for the separation and characterization of urine proteins include urine protein electrophoresis and urine protein immunofixation. Procedural details for both techniques are provided herein.

Key words: Urine analysis, Blood cancers, Plasma cell dyscrasia, Urine protein electrophoresis, Immunofixation

1. Introduction

1.1. Urinalysis

Urinalysis is the chemical and physical evaluation of urine and includes a broad spectrum of clinical assays looking for renal, urinary tract, and other disorders (1). It has a long rooted history and has been a method of health evaluation since ancient times (2).

In collecting urine it is important to preserve the composition, as it degrades very quickly upon excretion. The most common means of preservation is refrigeration, although this can cause precipitation of crystalline substances and proteins. In collecting urine, the easiest sample is a spot collection in which the patient collects an excretion at any random time. Spot collection is not as reliable as a timed collection due to the number of variables the

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urine composition is contingent upon, including hydration, diet, and/or whether it is the first morning's excretion. The latter is best for a spot collection because of the length of time (approximately 8 h) it has been collecting in the bladder, and thus the concentration of analytes is highest. A spot collection sample is useful in routine analysis, but for more specific tests, a timed collection is preferred. Timed collections are most commonly 24 h of urine, and depending on the tests to be performed (i.e., urine metanephrines or urine catecholamines), preservatives, such as 6N HCl, may be required. In the timed collection the first excretion of the first day is discarded and every excretion following is collected up to and including the first excretion of the next day. This collection method affords an accurate assessment of the patient's renal function and, subsequently, pathology. In a routine urinalysis physicians test for glucose, bilirubin, conjugated bilirubin, ketones, proteins, red blood cells, and the physical properties of the sample (1).

Analysis of urine is usually performed as an initial screen which is subsequently followed up with blood serum and/or other analyses. Urine is an ideal sample for screening since it is easy to collect, cost-effective, and can provide a wealth of information. The collection of the urine sample itself is a noninvasive process making it a better test for an initial or follow-up screen. The results from an urine analysis can include information regarding proteins, casts and monoclonal free light chains, enzymes and cell types present. Clinicians can perform a differential diagnosis or can narrow the investigation of diseases affecting a patient based on the combination of abnormalities detected in the aforementioned components of a urine sample. However, diagnosis of any condition cannot rely solely on urine analysis as the definitive determinant. Confirmatory tests of equal or greater specificity are used once the urine analysis has focused the investigation into a subset of conditions from which a patient may be suffering. For example, when looking at the protein content of a urine sample, there are some tests more commonly used than others. Although a physical evaluation can be done by visual inspection, it is only effective when the protein content of the urine is sufficiently elevated to be seen by the naked eye. Dipstick analysis can also provide qualitative and, sometimes, quantitative information on proteins and pH (3). The different reaction strips on a dipstick provide a more specific analysis than a visual evaluation. To further narrow an investigation, there are additional tests that are protein specific. These analyses include urine immunofixation (UIF) and urine protein electrophoresis (UPE), which can provide specific qualitative and quantitative results regarding free kappa and free lambda light chains. There are many disease states that are detectable in urine including diabetic nephropathy, glomerular

disease and other renal disorders, but for this chapter we will review various aspects of urine analysis with particular regard to the diagnosis of patients with malignancies.

1.2. The Kidney

The kidney is comprised of units called nephrons with each nephron comprised of several parts. The renal corpuscle is made up of the glomerulus and Bowman's capsule. The glomerulus is the site of protein filtration from the blood, whereas all other filtrates (small, low molecular weight proteins) enter Bowman's capsule. The filtration process in the glomerulus is based on size and charge with cations more apt to be filtered than anions. The glomerulus is capable of filtering 125 mL/min and catabolizing up to 30 mg of proteins/day. Resorption of necessary blood components into the bloodstream occurs through the semipermeable membranes of the tubules and also secretion of unnecessary blood particles into the tubule. If there is an excess of certain minerals beyond normal levels the tubules resorptive capacity is surpassed and such blood components as bilirubin, glucose, ketones can be deposited in the collection duct. All these materials, to be excreted from the body, are then deposited in the collection duct and leave the body as urine.

In a healthy adult human, a 24-h urine collection should contain <150 mg of protein/day. In the event that there is significantly more protein present in such a urine collection, renal problems should be suspected. In a normal urine sample there are detectable levels of creatinine and urea, but in the background of diseases such as myeloma or proteinuria, there are also detectable levels of proteins such as kappa and lambda free light chains.

In a disease state, renal function is interrupted in several ways, including loss of charge on the glomerulus allowing more proteins to filter through the glomerulus than would normally occur, or renal casts being formed in the tubules. In order for significant amounts of protein to be seen in a urine sample, the renal threshold must be exceeded. This threshold is defined as the point where tubular absorptive capacity is surpassed.

Normal production of free light chains is approximately 500 mg/day and the absorptive capacity in the glomerulus and tubules is 10–30 g/day. When blood serum concentration of monoclonal kappa free light chains is 6–7× the upper limit of the reference range, and in the case of monoclonal lambda free light chains, when the level reaches about 12–18× the upper limit of the reference range, the proteins are significantly detectable in urine. This condition is termed overflow proteinuria, and is often accompanied by genetic mutations causing defective antibody overproduction with kappa and lambda free light chains exhibiting extended half-lives. Overflow proteinuria is a sign of high serum protein levels and can be indicative of myeloma or a related disease. The excess protein filtering

thought the kidney adds strain to the glomerulus and organ as a whole. As the kidney begins to fail due to stress from formed casts and excess filtrates, disease becomes more apparent on UIF and UPE gels. Thus, a urine sample presents signs of disease, contingent upon the health of the kidneys, making it a useful indicator of disease state and diagnosis. The types of proteins of particular interest in urine analysis of cancer patients are referred to as Bence Jones proteins – these are monoclonal kappa and lambda free light chains, named after Dr. Henry Bence Jones.

Although Dr. Bence Jones was not the first to discover kappa and lambda light chains, he was the first to describe their importance in diagnosing myeloma (4). Thomas Watson and William Macintyre were the first to discover such proteins as a precipitate in their patient, Thomas Alexander McBean's, urine sample. Not knowing the identity of the precipitate that was formed upon warming, and disappeared when warmed further, they sent a sample of McBean's urine to Dr Henry Bence Jones.

Upon examination of McBean's urine sample, Bence Jones concluded that the precipitate was an "oxide of albumen" further described as "hydrated deutoxide of albumen". He then described a connection between McBean's autopsy results, which included such findings as soft/brittle bones filled with gelatinous substance and an abundance of plasma cells, and the protein in the urine. He is noted as saying "I need hardly remark on the importance of seeking for this oxide of albumen in other cases of mollities ossium". Bence Jones correctly posited that the urine precipitate was related to McBean's autopsy results, which would now indicate that McBean suffered from multiple myeloma. The connection Bence Jones made has proved crucial to testing and analysis used today in evaluating myeloma patients. Bence Jones' oxide of albumen is now known as monoclonal (kappa and lambda) free light chains. The conclusions made by Bence Jones allow physicians today the ability to use detection of kappa and lambda free light chains as a clinical diagnostic tool. The presence of free light chains in urine is ultimately the result of a process of an immune response that has gone awry.

1.3. B and T Lymphocytes

B and T lymphocytes are key mediators of the immune system. B cells are involved in the humoral immune response, where they produce antibodies, which "tag" antigens for destruction by macrophages or natural killer cells.

T cells are involved in the cell-mediated immune response, where an antigen presenting B cell activates the T-cell receptor causing cytokine release. Cytokines are environmental cues used by T cells to direct the immune response – they can cause proliferation of more T cells, attract macrophages, or cause T cells to differentiate into cytotoxic cells.

Defects in B and T cell differentiation and response are the cause of many cancers where cell proliferation occurs at an uncontrolled rate, referred to as B- and T-lymphocyte neoplasms.

B lymphocytes are white blood cells from bone marrow that become plasma cells when stimulated by the appropriate proteins from bacteria or viruses entering the body. Gram-negative bacteria have surface proteins and other molecules that govern the reactions of a cell to the environment. Components such as lipopolysaccharide (LPS) stimulate B cells. LPS is comprised of several moieties – the lipid component, Lipid A, is the toxic member controlling physiological response, and a separate polysaccharide component guides immunogenicity. LPS elicits B lymphocyte differentiation into plasma cells, ultimately causing production of plasma cells commensurate with the levels of the presented antigen.

B and T lymphocytes conspire to mount an immune response to foreign antigens. Once the B cell has engulfed the antigen, digested it, and is presenting fragments of the digested antigen on the MHC, a mature T cell is attracted. B cells produce antibodies against the specific antigen present in the same process in which T cells are activated and give off cytokines to direct the immune response in other capacities. Some cytokines cause maturation of additional T cells, some make the existing T cells cytotoxic, and others attract macrophages to engulf infected cells.

Antibodies are produced by the B lymphocytes to bind and mark each antigen specifically. Once the antibodies have marked the antigen, the foreign component is degraded by phagocytosis and is processed in the spleen or liver. The T-cell response addresses the already infected cells by producing cytotoxic T cells and attracting macrophages. In this coordinated effort, B and T lymphocytes rid the body of the antigen and infected cells to restore a normal state.

In the case of lymphomas, massive overproduction of abnormal plasma cells occurs upon stimulation of the B lymphocytes by a specific antigen. The stimulated B cells produce abnormal plasma cells or myeloma cells, resulting from infection by a virus or through oncogenic mutations they have acquired. An overproduction of myeloma cells leads to overproduction of antibodies, but like the plasma cells from which they arise, they are not healthy antibodies. The abnormal antibodies are overproduced with weak disulfide bonds holding the light and heavy chains together. The inability of the heavy and light chains to stay bound by the disulfide bonds lead to excess monoclonal kappa and lambda free light chains and free heavy chains (usually of IgG subclass, but may also be IgM, IgA, IgE, or IgD). Kappa monoclonal free light chains exist as monomers and lambda monoclonal free light chains exist as dimers. Kappa free light chain is produced at a rate roughly two times that of lambda free light chain. In a UIF it is more likely to see free kappa banding than free lambda.

Accumulation of these cells leads to decreased available space for healthy white blood cells, red blood cells, and platelets in bone marrow. Excess free heavy and light chains lead to tumors of the bones and soft tissue. These tumors are representative of increase in monoclonal (M) protein produced by the B cells. Each B cell responds to a specific antigen and produces a specific M protein to coincide with the antigen. Genetic mutations in the associate signaling pathway can cause plasma cells to continually produce the defective M proteins.

B-lymphocyte neoplasms include a long list of conditions from multiple myeloma to various leukemias and lymphomas. These are described further below. A paucity of healthy cells and growth of tumors weakening the hard bone is indicative of the condition referred to as hypercalcaemia. With this condition, there is increased levels of Ca^{+} in the blood and affects many organs including the kidney, GI, heart, and also the nerves and muscles. Polyuria will occur as the kidney is affected by the increase in Ca^{+} and other components in blood. The glomerulus and tubules of the nephrons suffer in an attempt to filter out excess proteins and Ca^{+}. Eventually the stress on the kidney from the excess proteins causes nephron failure and ultimately renal failure. The blood containing excess protein and Ca^{+} has similar effects on other organs.

1.4. Plasma Cell Disorders

1.4.1. Waldenstrom's Macroglobulinaemia

Waldenstrom's macroglobulinaemia is associated with any detectable IgM monoclonal gammopathy, specific surface proteins, and invasion of bone marrow, spleen, and lymph nodes by plasmacytoma prone lymphocytes. It tends to affect older men more than women and can be accompanied by secondary amyloidosis. Although it is possible to test for Waldenstrom's using urine, it is not the most accurate form of analysis due to polymerization of IgM and glomerular filtration of large molecular weight proteins. Bence Jones proteinuria does occur (often showing elevated free Kappa light chain) in about half the patient population, but is not indicative necessarily of the pathophysiology of the disease.

1.4.2. Plasmacytoma

Waldenstrom's macroglobulinaemia is not the only cancer characterized by plasmacytoid lymphocyte infiltrations into the bone marrow. Plasmacytoma is another form of cancer that can exist in two forms: solitary bone plasmacytoma (SBP) and extramedullary plasmacytoma (EMP). SBP is analagous to a solid tumor in myeloma dyscrasias since it is a single lytic bone lesion invaded by myeloma cells. It can be detected in about two-third of cases in UPE, but in one-third of cases both UIF and UPE should be used. It is otherwise characterized by localized bone pain caused by bone destruction usually in the axial skeleton (i.e., vertebrae) and an absence of symptoms of systemic MM. Tracking of urine

protein is not the best way to monitor SBP however. Urine analysis is best used as a diagnostic tool in this case. As for EMP, UIF or UPE are not ideally suited for its detection. It is an invasion of soft tissue by monoclonal plasma cells, most often in the head and neck areas.

1.4.3. Heavy Chain Disease

Heavy chain disease is identified as malignant plasma cells producing only incomplete monoclonal immunoglobulins without light chains being produced. There are three types of heavy chains considered in this disease, IgA, IgG, and IgM.

IgA heavy chain disease occurs primarily in a geographically localized area, the Middle East. It is thought to be caused by a parasite or other microorganism. IgA heavy chain disease occurs in individuals between ages 10 and 30 and is not detectable in urine. It is best identified in intestinal fluids.

IgG heavy chain disease can be asymptomatic and benign but is more often exhibited in malignant lymphoma. This heavy chain disease however can be detected in urine as it displays proteinuria as >1 g protein/24-h collection. Additionally, amyloidosis can develop as a secondary disease.

IgM heavy chain disease occurs in older adults and displays free kappa Bence Jones proteinuria in approximately 1% of patients. Serum analysis is normal or displays hypogammaglobulineamia. Death usually results, caused by uncontrolled proliferation of chronic lymphocytic leukemia (CLL) cells.

1.4.4. Amyloidosis: Primary Amyloidosis (AL), Secondary Amyloidosis (AA)

A detectable B-lymphocyte dyscrasia using UIF and UPE is AL amyloidosis. AL amyloidosis is caused by M protein (amyloid fibrils) deposits in specific organs (localized) or spread throughout the body (systemic). This conditions often leads to organ failure due to tissue damage caused by the amyloid deposits. In diagnosing AL amyloidosis UIF is a more sensitive assay to detect FLC initially and then serum analysis is best to quantify the findings. It is commonly found that MM patients develop AL amyloidosis resulting in renal casts derived from kappa free light chains. These dense protein deposits eventually cause organ failure and death.

Secondary amyloidosis (AA) is similar to AL, except that is it known to accompany other systemic infections and is not characterized as an independent disease. In contrast to AL, in AA the deposited proteins are secondary proteins whereas in AL, the deposited proteins are immunoglobulins. AA usually occurs when there is inflammation or tissue damage in the body. Upon inflammation or tissue damage acute phase reactants are activated to minimize the effects, but when the acute phase reactant serum amyloid A is degraded, it leaves AA proteins to make deposits in the body. This is not detectable in urine, but accompanies diseases such as MM, which are detectable in urine.

1.4.5. Multiple Myeloma: Non-secretory Multiple Myeloma

Multiple myeloma is characterized by an accumulation of myeloma cells in bone marrow, which ultimately leads to tumor growth in bones throughout the body. Multiple myeloma is identified as a monoclonal gammopathy in serum and/or urine. Additional identifiers include osteolytic lesions on the axial skeleton, plasmacytosis, anemia, renal failure, and hypercalcaemia. These types of symptoms are also indicative, potentially, of subclasses of multiple myeloma. For example, nonsecretory multiple myeloma is characterized by the same symptoms as multiple myeloma with the exception of a monoclonal gammopathy. This characteristic means that with such tests as UIF or UPE it would not be possible to detect any free light chains or heavy chains because the myeloma cells are not secreting immunoglobulins. This condition exists with two types of myeloma cells, "producer" type and "nonproducer" type, where producers make immunoglobulins that never pass through the cell membrane for an as of yet undetermined reason and nonproducers do not produce immunoglobulins at all. In such a situation, a clinician would use tests in combination with urinalysis in order to determine the disease, and would rule out standard multiple myeloma. Subsequent work-up (following urinalysis) may include bone marrow aspiration or immunoperoxidase staining to diagnose nonsecretory multiple myeloma.

1.4.6. Multiple Myeloma: Smoldering Multiple Myeloma/MGUS

Another subset of MM is smoldering multiple myeloma, which can best be discussed in combination with monoclonal gammopathies of undetermined significance (MGUS). MGUS is characterized as an intact monoclonal immunoglobulin in a patient showing no other signs of B-cell dyscrasias, <10% clonal plasma cells in bone marrow, and <30 g/L of myeloma cells. This is a very common plasma cell dyscrasia in the population over 50 years of age. It is not a life threatening condition, but is monitored closely due to the possibility of progressing to MM. About 1% of those with MGUS progress to malignant monoclonal gammopathies and most of those cases have IgM or IgA monoclonal gammopathies. In the case of MGUS, Bence Jones proteinuria can be an indication of progression to malignancy if the urine protein exceeds 50 mg/day. From MGUS, disease may progress to SMM and eventually develop to MM. SMM is characterized as 10% or greater clonal plasma cells in bone marrow and >30 g/L of myeloma cells. These patients do not have progressive MM; their disease is stable for an extended period of time during which treatment is not necessary, but abnormal laboratory results are constant. Urine Bence Jones proteins greater than 150 mg/day are a likely indication of SMM. Of the patients with IgA or IgM monoclonal gammopathies in MGUS, those with IgA are more likely to progress to Waldenstrom's macroglobulinaemia or lymphoma.

1.5. Analysis of Urine

1.5.1. Urine Immunofixation Electrophoresis

UIF is a method of resolving proteins of a concentrated urine sample in an effort to identify abnormalities or problems with kidney function. UIF is adapted from a serum IFE in-vitro diagnostic procedure, which is used to help identify such malignancies as MM or liver disease. In serum IF, the goal is to identify any monoclonal, polyclonal, or oligoclonal gammopathies; in UIF the goal is to detect the presence of kappa and lambda light chains in urine (see Figure 1).

Urine samples are only tested for presence of free light chains and serum is tested for the presence of both heavy and light chains. Urine results are indicative of renal failure and excess antibodies present in the blood stream, both of which are signs of oncological malignancies. Appearance of proteins such as monoclonal free light chains in a urine sample is indicative of the protein threshold in the blood being exceeded. The results are read from the gel in a qualitative manner and supplemented by serum Free Light Chain quantitation provided by the Beckman IMMAGE automated system (Beckman Coulter, USA). UIF can be performed regardless of the urine total protein result. Often, UIF is ordered in combination with a UPE. Interpretation of UIF is subjective as it is relative to a set of controls producing banding of fixed intensity.

1.5.2. Urine Protein Electrophoresis

UPE is performed similarly to UIF in that it is also a concentrated urine sample electrophoresed on similar agarose gels, but there is one lane per patient and no antisera are applied, the sample is simply run to resolve the proteins based on charge (see Figure 2). The gel is then scanned and the various protein bands are

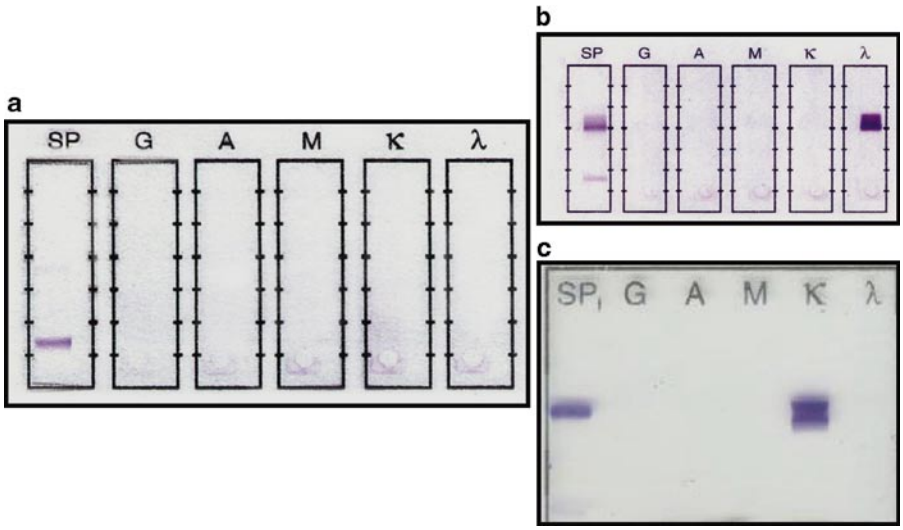

Fig. 1. Urine immunofixation electrophoresis. (**a**) normal pattern from patient urine. (**b**) free lambda light chain. (**c**) free kappa light chain.

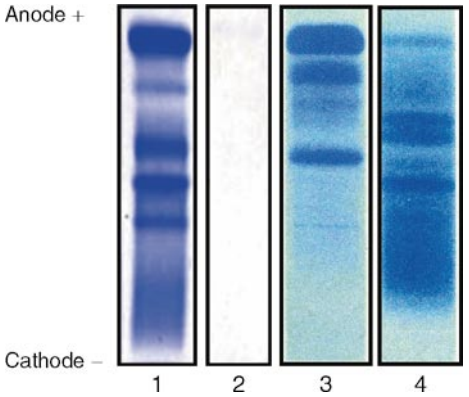

Fig. 2. Urine protein electrophoresis patterns relative to serum proteins. Lane 1, serum proteins; lane 2, urine proteins from normal patient; lane 3, urine proteins from patient with glomerular proteinuria; lane 4, urine proteins from patient with tubular proteinuria. Note that in normal patient, very little protein is detected. Furthermore, the composition of proteins in glomerular (where high molecular weight proteins dominate) is very different from that of tubular proteinuria (low molecular weight proteins dominate).

quantified based on the total protein. If the total protein of a urine sample is less <10 mg/dL, UPE is not performed as the pattern is unable to be differentiated.

Interpretation of PE/UPE results is done after scanning and quantitation is added based on the urine total protein. In the case of a UPE the bands are as follows from left to right: Albumin, Fraction 2, Fraction 3, Fraction 4, and Fraction 5. Abnormal banding patterns in combination with abnormal quantitation can indicate malignancies. Most commonly abnormal banding occurs in the F4 and F5 regions.

From these diseases, the importance of urine analysis in diagnosing and assessing treatment response for several plasma cell dyscrasias is evident. Differential diagnosis entails narrowing the realm of possible afflictions so clinicians can decide on the next step in treating a patient. As a secondary tool urine analysis is helpful in determining stage of disease as in multiple myeloma. Many times the result of urinalysis is the result of kidney status, which is strongly affected by B-lymphocyte dyscrasias. Urine analysis is useful also as a follow up test to monitor FLC levels and disease progression. Although is not highly sensitive, it is cost-effective and a good screening procedure for new patients. It is best used in combination with serum IF and serum PE, and is invaluable because of its ease in sample acquisition and analysis. UIF and UPE have proven to be useful tools in monitoring many cancers, but as the demand for more sensitive and specific tests increases, urine analysis may ultimately become an adjunct procedure.

2. Materials

1. SPIFE 3000 (Helena Laboratories, Beaumont, TX).
2. QuickScan 2000 (Helena Laboratories, Beaumont, TX).
3. SPIFE SPE gels (catalog # 3422, Helena Laboratories, Beaumont, TX).
4. SPIFE Immunofix gels (catalog # 3409, Helena Laboratories, Beaumont, TX).
5. Acid Violet Stain (Helena Laboratories, Beaumont, TX); dissolve powder in 1 L of 10% acetic acid.
6. Acid Blue Stain (Helena Laboratories, Beaumont, TX); dissolve powder in 1 L of 5% acetic acid and stir 30 min prior to use.

3. Methods

3.1. IFE – Electrophoresis Chamber

1. Remove gel (IFE 9 or IFE 6) from the packaging and place on the urine IFE template board. Blot off excess preservative with SPIFE Blotter A. Remove and discard blotter.
2. Select appropriate kit for gel size, 9 or 6 samples. Place three red disposable application templates over the gel and on the pegs on either side of the template board. The corner with the hole should be on the lower left position of the applicator template and the wells should line up with the lanes of the gel.
3. Apply 3 μL of concentrated urine to the kappa and lambda lanes of each sample block. Use the first gel block for Free Kappa and Free Lambda controls. (i.e., each IFE 9 has one control block and eight patient blocks and IFE 6 has one control block followed by five patient blocks).
4. Time sample application for 5 min; blot with blotter A-plus.
5. Remove disposable application templates.
6. Pipette 2 mL of REP-Prep buffer onto the electrophoresis surface of the SPIFE 3000. Place the round hole of the gel onto the round peg at the left of the chamber and lay over the REP-Prep, making sure no bubbles are under the gel as they will result in problems with electrophoresis. Blot any excess REP-Prep from the edges of the gel with a lint free cloth.
7. Place the carbon electrodes on the gel blocks and touching the outside of the magnetic pegs. Close the lid to the electrophoresis chamber.

8. Select SERUM IFE from the test list by hitting Test Select on both the electrophoresis chamber and the staining chamber. On the electrophoresis side hit Start/Stop followed by Test Select/Continue until the screen reads ELECTROPHORESIS; hit Start/Stop to begin gel electrophoresis.
9. The SPIFE 3000 will indicate when electrophoresis is complete and the antisera need to be applied. Remove carbon electrodes and gel blocks.
10. Choose the appropriate antisera template (IFE 9 or 6) place the round hole over the round peg on the left of the chamber. Press down gently to ensure a good seal for each lane over the gel. Using a pipette set to 50 μL, manually dispense free kappa and free lambda antisera into the hole at the right side of the appropriate lanes.
11. Close the chamber lid and press Test Select/Continue to begin antisera application.
12. The next indication beep will signify need to blot excess antisera using the blotter combs for Blot 1 and Blotter D for Blot 2. After placing each blotter in the antisera template and under the antisera template respectively hit Test Select/Continue to time each blot.
13. Following Blot 2, remove the antisera template and replace the electrodes on the outside of the magnetic pegs to predry the gel.

3.2. IFE – Staining Chamber Preparation

1. Select SERUM IFE from the test list. Press Start/Stop to begin chamber preparation approximately 3 min before completion of electrophoresis. Press Start/Stop again followed by Test Select/Continue indicating that the wash container is full and the chamber can be prepared properly.
2. When the chamber is prepared the machine will indicate completion and the screen will read WASH 2. Remove the gel holder until the gel itself is ready to be put in for staining.

3.3. IFE – Staining Chamber

1. With electrophoresis complete and the staining chamber prepared attach the gel to the Gel Holder by the round hole on the peg of the left arm of the gel holder, the middle of the gel under the middle arm and oval hole on the peg of the right arm. Make sure the gel is facing away from you and the middle arm of the gel holder is touching the back of gel.
2. Insert the gel, still facing away from you, into the staining chamber. Press Start/Stop to continue in WASH 2 and through staining, destaining and drying.

3. Upon completion in the staining chamber the gel holder and gel can be removed and the gel scanned using the QuickScan 2000.

3.4. UPE – Electrophoresis Chamber

1. Remove gel (IFE 9 or IFE 6) from the packaging and place on the urine PE template board. Blot off excess preservative with SPIFE Blotter A. Remove and discard blotter.
2. Select appropriate kit for Urine Protein. Place the red disposable application template over the gel and on the pegs on either side of the template board. The corner with the hole should be on the lower left position of the applicator template and the wells should line up with the numbered lanes of the gel.
3. Apply 2 µL of concentrated urine to each well of the template. Use the first two lanes for normal and abnormal controls (Level 1 and Level 2). The controls are run at a 2× dilution. Time sample application for 5 min; blot with blotter A-plus.
4. Remove disposable application template.
5. Pipette approximately 2 mL of REP-Prep onto the electrophoresis surface of the SPIFE 3000. Place the round hole of the gel onto the round peg at the left of the chamber and lay over the REP-Prep, making sure no bubbles are under the gel as they will result in problems with electrophoresis. Blot any excess REP-Prep from the edges of the gel with a lint free cloth.
6. Place the carbon electrodes on the gel blocks and touching the outside of the magnetic pegs. Close the lid to the electrophoresis chamber.
7. Press Test Select/Continue until SERUM PROTEIN appears on the screen. Press Start/Stop to begin electrophoresis.
8. After electrophoresis, remove gel blocks and replace electrodes for predry.

3.5. UPE – Staining Chamber

1. Remove the Gel Holder and attach the round hole of the gel on the left arm peg and the oval hole onto the peg of the right arm making sure the middle arm of the Gel Holder is touching the back of the gel. With the gel facing away, insert the gel and gel holder into the staining chamber. No preparation is necessary for PE/UPE.
2. Press Test Select/Continue until the screen reads SERUM PROTEIN.
3. Press Start/Stop once. At the next prompt press Start/Stop again to begin staining.
4. Upon completion in the staining chamber the gel holder and gel can be removed and the gel scanned using the QuickScan 2000.

4. Notes

1. Unlike serum samples, urine samples may be dilute and should be concentrated using microconcentrators (Amicon) prior to analysis.
2. Urine samples can be concentrated according to the following stipulations:

Protein (mg/dL)	Concentration factor
<50	100×
50–100	50×
100–300	25×
>600	5×

3. If the urine sample is cloudy, it should be first centrifuged at 2,000 × *g* for 5 min, to remove any solid precipitates that would interfere with concentration and analysis.
4. It is common to run a single electrophoresis gel with both serum and urine samples, with the different sample types segregated in different rows.

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