METHOD AND DEVICE FOR TESTING FOR BENCE-JONES PROTEIN

A method, device, and various kits for a specific binding assay is provided for the detection of free light chains in untreated urine. Such methods, devices or kits may be used for screening for a disease state producing bence jones protein, comprising testing for bence jones protein presence in unspun or un-centrifuged urine. Such methods, devices or kits may be for monitoring for and adjunctive testing of such disease states. An in vitro device (IVD) may provide for detection of unspun urine light chains (BJP). Such device may be an IVD test assay, a tube (which may be used in a clinical laboratory or doctor’s office, among others) incorporating a dipstick, or a cassette using lateral flow technology (which cassette may be used for home testing or point-of-care testing, among others). The device is effective for any or all of the following with regard to the discussed disease states, which produce BJP detectable by the device: screening, monitoring and adjunctive testing. Accordingly, the present disclosure provides a method of screening for a disease state producing BJP, comprising testing for BJP presence in unspun/un-centrifuged urine. Similar methods for monitoring for and adjunctive testing of such disease states are also provide herein.
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— of inventorship (Rule 4.17(iv)) for US only

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the “Guidance Notes on Codes and Abbreviations” appearing at the beginning of each regular issue of the PCT Gazette.
METHOD AND DEVICE FOR TESTING FOR BENCE-JONES PROTEIN

BACKGROUND AND FIELD

[0001] The present disclosure relates generally to assay devices and specifically to those devices making use of chromatographic techniques in conducting specific binding assays for the determination of the presence of light chains (free & bound) in untreated human urine.

[0002] Tests for determining the presence of light chains (free and bound) are presently known in the art, as is the use of chromatographic strips for the detection of protein. The use of such chromatographic strips for the detection of light chains however has not been proposed or used for the detection of light chains in untreated urine before the filing of co-pending U.S. Patent Application Serial No.09/823,183, filed March 30, 2001, the entire contents of which are specifically incorporated herein by reference. Co-pending U.S. Patent Application Serial No. 10/202,587, filed July 24, 2002 and directed to a testing cassette, and co-pending U.S. Provisional Patent Application Serial No. 60/573,914, filed May 24, 2004, are also specifically incorporated herein by reference. The present disclosure refines the problems of insensitivity, unreliability, high cost, and time consumption of the typical state of the art tests, as presently known, and applies such testing as indicators for various disease states.

[0003] The present disclosure describes an improved, less expensive, easy to use test for the detection of free and bound light chains, as well as simplified test procedures without the prior problems of known tests which can be laborious, time consuming, costly, and require skill in interpretation. The chromatographic method and devices of the presently described devices are capable of reliably sensing free and bound light chains with increased sensitivity and require minimal interpretation. The improved method and devices described herein thereby enable physicians and assistants, and indeed, home users thereof the ability to routinely perform such a test for the detection of free light chains in a doctor's office or in a home without the wasted time and expense of sending urine samples to a lab for laborious examination. In cases where the labor-intensive state of the art tests have been performed, the claimed invention can also be used to quickly verify any results.
[0004] It is known that an immunoglobulin is schematically made up of two heavy chains and two light chains. Determinations of the presence of the free light chains, also called Bence Jones proteins, which pass into the urine is of great interest from a diagnostic viewpoint.

[0005] Gammopathies characterized by the proliferation of B cell clones, yield increased clonal production of immunochemicals. In addition to the primary effects of the gammopathy, secondary consequences dependent on the class of clonal immunochemical produced may emerge. The preferential production of single immunoglobulin clones will result in immunodeficiency as a result of normal immunoglobulin production, and the production of toxic free light chains will cause renal disease even at low-level deposition. Immunological pathologies caused by the presence of free light chains in the urine, which is the consequence of the increase thereof in the blood, is associated with and can be summarized as (a) the presence of monoclonal free light chains, i.e. immunoproliferative illnesses such as multiple myeloma, micromolecular myeloma, Waldenström's macroglobulinemia, chronic lymphatic leukemia and primitive amyloidosis; and (b) the presence of polyclonal free light chains, i.e. hyperimmune illnesses such as systemic lupus erythematosus, acute rheumatoid arthritis and secondary amyloidosis. Additional immunological pathologies presenting free light chains in urine may include, but are not limited to, monoclonal gammopathies of undetermined significance (MGUS), Fanci’s Syndrome, renal insufficiency/renal failure, renal failure due to diabetes, and renal failure due to hypertension.

[0006] Classification of monoclonal gammopathies, as described by Gertz and Greipp. Ch. 1, Table 1.1, from Kyle, 2002, Rev Clin Exp Hematol, includes I), monoclonal gammopathy of undetermined significance (MG-US) and II) malignant monoclonal gammopathies. Monoclonal Gammopathy of Underdetermined Significance (MG-US) are generally benign (IgG, IgA, IgD, IgM, and rarely Free Light Chains). Association with neoplasia of cell types is known to produce M proteins. Such man include bicalonal gammopathies and idiopathic bence-jones proteinuria.

[0007] Malignant monoclonal gammopathies include, without limitation, multiple myeloma (MM) (IgG, IgA, IgD, IgE, and free κ or λ light chains), including: overt MM;
smoldering MM; plasma cell leukemia; nonsecretory myeloma; IgD myeloma; POEMS: polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes (osteosclerotic myeloma); plasmacytoma; malignant lymphoproliferative disorders; Waldenstrom’s macroglobulinemia; malignant lymphoma; chronic lymphocytic leukemia; heavy-chain diseases; amyloidosis; primary amyloidosis; and amyloidosis with MM (secondary, localized and familial amyloidosis have no M protein).

[0008] Monoclonal gammopathies are generally a group of medical (clinical) disorders/diseases characterized by the proliferation of a single clone of plasma cells (PC), which produce a homogeneous monoclonal protein (M protein). Such monoclonal gammopathies represent approximately 10% of hematologic malignancies. Only 1-2% of patients are seen by hematologist or oncologist, and in the Mayo Clinic experience, there are generally 5,000 patients per year.

[0009] As drawn from “Clinical Diagnosis And Management Of Patients With Multiple Myeloma”, Clinical Diagnosis & Management by Laboratory Methods, John Bernard Henry, M.D., Multiple Myelome (MM) is a disease caused by neoplastic proliferation of abnormal plasma cells (myeloma cells), primarily occurring in the bone marrow. MM is rare under the age of 40. The mean age at the time of diagnosis is 62 years. The incidence of this disease is equal in men and women. Bone pain is the most common symptom, and pathological fractures are frequent. The growth of myeloma cells in the marrow produces multiple tumors, which appear on x-ray as multiple punched-out osteolytic lesions of bone destruction; occasionally the growth is diffuse and appears as diffuse osteoporosis. An unusual propensity to infection is common because of impaired production of antibodies.

[0010] Plasma cells are a special type of white blood cell that is part of the body’s immune system. Plasma cells normally line in the bone marrow and make proteins, called antibodies, which circulate in the blood and help fight certain types of infections. Plasma cells also play a role in the maintenance of bane, by secretion of a hormone, called osteoclast activating factor, which causes the breakdown of bone. Patients with MM have increased numbers of abnormal plasma cells that may produce increased quantities of dysfunctional
proteins detectable in the blood and/or urine. These abnormal proteins are referred to as paraproteins or monoclonal proteins in the blood (M proteins) or urine (Bence Jones protein). In MM, plasma cells infiltrate the bone marrow, spreading into the cavities of all the large bones of the body. As the number of plasma cells increases and greater amounts of proteins are produced, patients experience decreased bone marrow blood cell production, fragility of the bones, pain and abnormal kidney function. They may also have decreased quantities of normal antibodies necessary to fight certain types of infection. TABLE 1, below details common symptoms in patients with Multiple Myeloma:

**Table I. Common Symptoms in Patients with Multiple Myeloma**

<table>
<thead>
<tr>
<th><strong>Symptom</strong></th>
<th><strong>Description and cause</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney Problems</td>
<td>Excess protein in the blood, which is filtered through the kidneys. Can cause kidney damage and lead to renal failure. Increased calcium in the blood (hypercalcemia) overworks the kidneys and can cause a variety of symptoms, including loss of appetite, fatigue, muscle weakness, restlessness, difficulty in thinking or confusion, constipation, increased thirst, increased urine production, and nausea and vomiting.</td>
</tr>
<tr>
<td>Bone Pain</td>
<td>Pain in the lower back or in the ribs. This is the result of tiny fractures in the bones caused by accumulation of plasma cells and weakened bone structures.</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Abnormal/malignant plasma cells expand in the bone marrow causing red blood cell production to be suppressed, leading to anemia. Anemia can cause unusual tiredness and abnormal paleness.</td>
</tr>
<tr>
<td>Recurrent infection</td>
<td>Increased numbers of myeloma cells can also suppress the production of infection-fighting white blood cells, leading to reduced immunity and the possibility of frequent recurrent infections, such as bacterial pneumonia, urinary-tract infections, and shingles.</td>
</tr>
</tbody>
</table>

[0011] Multiple myeloma may be diagnosed according to any of the following indicators: 1) Osteolytic “punched-out” lesion on bone x-ray; 2) Abnormal, morphologically bizarre,
malignant-appearing plasma cells in the bone marrow; and 3) Bence Jones proteins (light chains of immunoglobulin) in the urine.

[0012] Blood in a patient with MM may indicate normochromic normocytic anemia; and normoblasts may be present in the blood. Typically, the leukocyte count is slightly decreased, normal, or slightly increased. The platelet count is usually normal, but may be decreased. The most striking features of the blood is the marked degree of rouleau formation, which may make cell counting difficult.

[0013] Bone marrow in a patient with MM may indicate the presence of at least 10 percent of the bone marrow nucleated cells being plasma cells. Dissociation of nuclear and cytoplasmic maturaron is a distinctive feature of the myeloma cells.

[0014] With regard to immunoglobulins in a patient with MM, Serum globulin is usually increased, often strikingly so. This increase is responsible for the tendency toward rouleau formation and an elevated erythrocyte sedimentation rate (ESR). Immunoelctrophoresis indicates that the monoclonal protein is IgG in over half the cases of MM, IgA in about on fifth, IgD in less than 1 percent and IgE very rarely. In each of these groups of MM, some patients secrete light chains (kappa of lambda) in addition to the whole immunoglobulin molecule. In about one quarter of the patients with MM, only light chains (Bence Jones protein) are produced by the abnormal plasma cells. Hypogammaglobulinemia is found in the latter group because light chains are filtered through the real glomerulus, leaving little or none in the serum, in addition to the fact that immunoglobulin production by the non-malignant plasma cells is greatly reduced in all patients with MM. Roughly 5 percent of myeloma proteins are cryoglobulins-that is, proteins that precipitate from cooled serum and redissolve on warming. Proteinuria is frequently present in multiple myeloma. Amyloidosis, which is present in about 10 to 15 percent of cases of MM may be a factor in the renal failure. Amyloid fibrils in cases of myeloma appear to have as their major protein component the light chains of immunoglobulin molecules.

[0015] The stages of multiple myeloma are as follows: Stage I) Tests indicate a low tumor volume. Stage I is divided into 2 groups: A) **Monoclonal Gammapathy of Undetermined**
Significance (MGUS): The M-protein is less than 3 grams per deciliter and less than 10% of cells in the bone marrow are plasma cells, with no other abnormalities; and B) Smoldering Myeloma: The M-protein is over 3 grams and more than 10% of the cells in the bone marrow are plasma cells. Lab Values for Stage I are: M protein IgG less than 5.0 gm/100 ml serum; IgA less that 3.0 gm/100 ml serum or urine Bence Jones protein less than 4 gm in 24 hours; normal serum calcium, normal bones and hemoglobin over 10.0 gm/100 ml serum.

[0016] Stage II evidences an intermediate tumor volume. Lab values are between Stage I and Stage III.

[0017] For Stage III, tests indicate a high tumor volume. Lab values are: M protein IgG greater than 7.0 gm/100 ml serum; IgA greater than 5.0 gm/100 ml serum; urine Bence Jones protein over 12.0 gm in 24 hours; 3 or more bone lesions; hemoglobin less than 8.5 gm/100 ml serum or calcium over 12.5 gm/100 ml serum.

[0018] Additionally, multiple myeloma may recur or relapse. In such case, the multiple myeloma has persisted or recurred/relapsed following treatment with radiation and/or chemptherapy.

[0019] Treatment for MM rarely produces a cure. The goal of therapy is to control symptoms and slow disease progression. Without treatment, average survival time is 1.5 yrs. With aggressive treatment, younger patients may have survive 4 to 6 yrs. Survival depends both on treatment and the stage of the cancer at diagnosis.

[0020] Generally speaking, bence-jones protein-quantitative tests measure for the presence of Bence-Jones Proteins (free immunoglobulin light chains) in urine. The current methods of BJP detection from urine specimens rely on electrophoresis. Direct Urine Protein electrophoresis exploits the different electrophoretic motilities of proteinuria classes. A single pronounced electrophoretic band in the gamma or beta region is indicative of a monoclonal residue. This may be further classified by referral to immunofixation electrophoresis, where electrophoresis is followed by immunoprecipitation with immunoglobulin and light chain specific antisera.
[0021] Table 2, below, generally describes approaches, advantages and disadvantages of clinical assays for proteins in urine:

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Urine Protein/ Aulfosalicylic acid test (SSA)</td>
<td>Simple; Inexpensive; Commonly Used; measures all proteins-albumin, glycoprotein and immunoglobulin (Bence-Jones)</td>
<td>Poor sensitivity for free light chains</td>
</tr>
<tr>
<td>Urine Reagent Dipsticks</td>
<td>Simple, Inexpensive; Commonly Used; Detects albumin</td>
<td>Will not detect abnormal proteins including Bence-Jones</td>
</tr>
<tr>
<td>Urine Protein Electrophoresis</td>
<td>Simple; Manual/Semi-automated; Inexpensive; Monoclonal bands visualized; Sensitive in concentrated urine (10 mg/L); Scan top obtain quantitative results</td>
<td>Subjective Interpretation; Specimen concentration may result in protein loss; False bands can appear from concentration; Problems with specimen with heavy proteinuria and 24-hr collections</td>
</tr>
<tr>
<td>Immunofixation electrophoresis on urine</td>
<td>Commonly used; Very sensitive for concentrated urine (2-5 mg/L)</td>
<td>Non-quantitative; Expensive antisera; Slow; Technically difficult</td>
</tr>
</tbody>
</table>

[0022] The problem with the current state of urine testing for BJP is reflected in the following statement: “Immunofixation electrophoresis, where electrophoresis is followed by immunoprecipitation with immunoglobulin and light chain class specific antisera...is technically difficult, slow, expensive, and with a sensitivity below that specified as clinically significant
(typical gel electrophoresis sensitivity is 30-50 mg/L, it is recognized that 10 mg/L BJP is significant*). [Hobbs, 1967] Indeed, BJP may go undetected in the urine when immunofixation electrophoresis is negative and patient is not symptomatic.

[0023] The presence of free light chains in urine presupposes their anomalous increase in the serum of the subject but, given their low molecular weight, free light chains pass through the glomerular filter and do not persist in the blood. It is therefore necessary to perform an indirect investigation, ascertaining their presence in the urine.

[0024] Known diagnostic methods based on ascertainment of free light chains in the urine are of great interest but at present are blocked by the difficulties of performance of such an investigation. Current techniques such as that described in U.S. Pat. No. 5,141,877 to Massaro, herein incorporated by reference, necessarily require concentration of the sample because of the relatively small percentage of free light chains in the organic liquid even with serious pathological conditions of the subject. Electrophoretic examination of the unconcentrated sample results in unacceptably low sensitivity and the resulting unreliability. The time necessary for concentration is added however to the time required for electrophoretic analysis with the obvious drawbacks. The analysis performed on the concentrated samples undoubtedly raises the reliability of the results without however achieving reasonable certainty. On the samples which prove suspect under electrophoresis it is therefore very advisable to perform immunofixation or immuno-electrophoretic tests, the laboriousness and cost, and requirement for interpretation of which are known, to achieve truly satisfactory levels of sensitivity and hence reliability of the analysis results.

[0025] Of interest to the present application is the disclosure of U.S. Pat. No. 4,727,037, issued to Ring, relating to a method for determining the class and subclass of a test antibody which demonstrates adsorbing on nitrocellulose paper or diazo paper in a predetermining spatial arrangement, antisera directed to isotype class and subclasses of an immunoglobulin; contacting said adsorbed isotype specific antisera with a fluid containing a test antibody to form a complex between the specific immunoglobulin heavy and light chains of the adsorbed antisera and said
test antibody present in the fluid; and reacting the complex so formed with a chromogenic substrate to thereby identify the specific class or subclass of said test antibody.

[0026] U.S. Pat. No. 5,141,877 describes a method for the determination of the presence of free light chains (Bence Jones protein) in a urine sample in which the sample is reacted with an anti-free light chain antiserum reagent, where the presence of the free light chains is revealed by increase in turbidity of the reacted sample. By comparison with the turbidity of calibrators having predetermined concentrations reacted with anti-free light chain antiserum, a quantitative analysis of the amount of free light chains in the urine sample can be determined. The test samples are derived from patients having secretory micromolecular myeloma.

[0027] U.S. Pat. No. 5,569,608 describes a method for determining the concentration of analyte in a test fluid by immunochromatography techniques which involves quantitatively determining the signals from captured analyte/labeled binding partner complex by an instrument, e.g. a reflectance spectrometer. A reflectance reading is determined for the captured complex and uncomplexed labeled binding partner which is captured in a separate zone of the immunochromatographic strip and the ratio of these reflectances is used to provide additional quantification to the assay method.

[0028] U.S. Pat. No. 5,780,308 teaches a multizone test device for semiquantitatively determining the presence of at least a predetermined minimum concentration of an analyte in a test sample. The device utilizes a strip of porous material, said strip comprising a reagent zone and a capture site. The reagent zone is upstream from the capture site. The reagent further contains a soluble conjugate comprising a labeled analyte-specific binding member which binds the analyte to form a labeled analyte complex. In addition the reagent zone has a capture reagent comprising an unlabeled specific binding member attached to the porous material. The capture reagent binds the labeled analyte complex to form an immobilized labeled analyte complex. Further the reagent zone includes a soluble calibration reagent comprising an unlabeled specific binding member which blocks the binding of the analyte to the capture reagent, thereby controlling the proportion of the analyte that binds to said capture reagent. As a consequence the analyte in the test sample must exceed a minimum concentration before the immobilized labeled
complex is formed. The calibration reagent is contained in said reagent zone. The capture reagent is immobilized at the capture site where the immobilized labeled complex is separated from the test sample, and where the presence of label associated with said immobilized labeled complex is detected to determine the presence of at least a predetermined minimum concentration of an analyte in the test sample.

[0029] U.S. Pat. No. 5,989,921 relates to a test device for determining the presence of a ligand in a liquid sample, the device has a casing defining an inlet and at least one window for viewing through a wall of said casing, a test strip disposed within said casing comprising a sorbent material which defines a flow path for transporting the liquid sample there along from said inlet to a test site and a control site, and, disposed upstream of said test site and said control site. Further this is a conjugate comprising a specific binder for the ligand and a colored particulate material. The test site comprises an immobilized first binding protein which binds specifically to the ligand, if the ligand is present in the liquid sample. The control site comprises an immobilized binder which binds said conjugate. The inlet, test site, and the control site are in lateral flow fluid communication along a flow path, such that after a liquid sample suspected to contain the ligand is applied to said inlet, said conjugate moves along said flow path and binds to said immobilized binder of said control site to produce a color visible to the unaided eye through at least one window indicative of a valid test result. If the ligand is present in the liquid sample, a specific binding reaction product comprising the ligand and said conjugate binds to the immobilized first binding protein of the test site to produce a color visible to the unaided eye through at least one window indicative of the presence of the ligand in the sample.

[0030] As stated above, known techniques are technically difficult, slow, and expensive and with a sensitivity below that specified as clinically significant (typical gel electrophoresis sensitivity is 30-50 mg/L, it is recognized that 10 mg/L BJP is significant). Since the presence of Bence Jones protein in the urine may not be supported or suggested symptomatically, a large percentage of positive samples remain unidentified in the early stages. The present invention relates generally to assay devices and specifically to those devices making use of chromatographic techniques in conducting specific binding assays for the determination of the
presence of free light chains in untreated urine. In particular, this technique provides kits for rapid determination between whole antibody and free light chain, and between classes of light chain. The present invention provides improved assays that require little technical expertise, are rapid, highly sensitive and may be used either as a screen, or as a confirmatory step prior to immunoelectrophoresis, or immunofixation electrophoresis utilizing untreated human urine.

SUMMARY

[0031] The present disclosure provides a method, device, and various kits for a specific binding assay for the detection of free light chains in untreated urine. Such methods, devices or kits may be used for screening for a disease state producing bence jones protein, comprising testing for bence jones protein presence in unspun or un-centrifuged urine. Such methods, devices or kits may be for monitoring for and adjunctive testing of such disease states.

[0032] In one embodiment, an in vitro device (IVD) provides for detection of unspun urine light chains (BJP). Such device may be an IVD test assay, a tube (which may be used in a clinical laboratory or doctor's office, among others) incorporating a dipstick, or a cassette using lateral flow technology (which cassette may be used for home testing or point-of-care testing, among others).

[0033] The device is effective for any or all of the following with regard to the discussed disease states, which produce BJP detectable by the device: screening, monitoring and adjunctive testing. Accordingly, the present disclosure provides a method of screening for a disease state producing BJP, comprising testing for BJP presence in unspun/un-centrifuged urine. Similar methods for monitoring for and adjunctive testing of such disease states are also provided herein.

[0034] In another embodiment, the device comprises a chromatographic immunoassay for detection of free light chains in non-centrifuged or un-concentrated/un-treated urine.

[0035] In another embodiment, a rapid chromatographic test is provided for detection of free light chains in urine, wherein the rapid test produces results within 5 minutes, with a
sensitivity greater than about 88% (for approximately 10mg/L urinary free light chain) and a specificity of greater than about 99%.

[0036] In another embodiment, a rapid chromatographic test is presented for detection of free light chains in urine, including a reaction device for kappa, and a reaction device for lambda.

[0037] In another embodiment, the present disclosure presents a method of diagnosing an indicator for a medical condition precipitating free light chains in urine, the method comprising exposing a chromatographic test device to untreated/unspun urine, wherein the chromatographic test device indicates presence of said free light chains in the urine. The medical condition may be one or more of: Multiple Myeloma, micromolecular myeloma, Waldstrom’s Macroglobulinemia, Chrimic Lymphatic Leukemia, Amyloidosis, lupus erythematosus, Monoclonal Gammopathies of Undetermined Significance, Fconi’s Syndrome, Renal Insufficiency/Renal Failure, Renal Failure due to Diabetes, Renal failure due to hypertension, and acute rheumatoid arthritis. The free light chain may be one or more of a monoclonal free light chain and a polyclonal free light chain.

[0038] In another embodiment, the present disclosure provides a cartridge, comprising at least one lateral strip pathway. In such cartridge, the lateral flow may begin at one origin (sample application) and proceed laterally along at least two distinct pathways.

[0039] In another embodiment, a home test cartridge is provided, including a conjugate pad or a lateral flow dipstick.

[0040] The above-discussed and other features and advantages of the present invention will be appreciated and understood by those skilled in the art from the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] Referring to the FIGURES wherein like elements are numbered alike in the several FIGURES:
[0042] FIG. 1 is a front plan view of one form of the test strip device;

[0043] FIG. 2 is a cross-sectional view of one form of the test strip device shown in FIG. 1;

[0044] FIG. 3 is a front plan view of one form of the test strip device of the present invention;

[0045] FIG. 4 is a front plan view of one form of the test strip device of the present invention showing the front face of the invention;

[0046] FIG. 5 is an exploded view of one form of the test strip device showing the front face of the invention;

[0047] FIGS. 6a and 8a are front plan views of two different forms of test devices in accordance with the present disclosure;

[0048] FIGS. 6b and 8b are cross-sectional views of the test devices shown in FIGS 1a and 3a respectively;

[0049] FIGS. 6c and 8c are front plan views of a kit embodiment of the present invention showing the test device shown in FIGS. 1a and 3a;

[0050] FIG. 7 is a schematic view of a sandwich type test device of the present invention; and

[0051] FIG. 9 is a schematic view illustrating how to interpret results of the competitive assay of the present invention.

**DETAILED DESCRIPTION**

[0052] As described above, the present disclosure provides a method, device, and various kits for a specific binding assay for the detection of free light chains in untreated urine. Such methods, devices or kits may be used for screening for a disease state producing bence jones
protein, comprising testing for bence jones protein presence in unspun or un-centrifuged urine. Such methods, devices or kits may be for monitoring for and adjunctive testing of such disease states.

[0053] In one embodiment, an in vitro device (IVD) provides for detection of unspun urine light chains (BJP). Such device may be an IVD test assay, a tube (which may be used in a clinical laboratory or doctor’s office, among others) incorporating a dipstick, or a cassette using lateral flow technology (which cassette may be used for home testing or point-of-care testing, among others).

[0054] The device is effective for any or all of the following with regard to the discussed disease states, which produce BJP detectable by the device: screening, monitoring and adjunctive testing. Accordingly, the present disclosure provides a method of screening for a disease state producing BJP, comprising testing for BJP presence in unspun/un-centrifuged urine. Similar methods for monitoring for and adjunctive testing of such disease states are also provided herein.

[0055] Clinical applications of free light chain immunoassays, such as is described herein includes, without limitation: plasma cell dyscrasia (PCD)(B-Cell malignancy); dyspreteinemia (Center of Excellence-Mayo Clinic); monoclonal gammopathies; monoclonal gammopathies of undetermined significance (MGUS); multiple myeloma; amyloidosis; and waldenstrom macroglobulinemia. Clinical utility of the present test includes: diagnosis and management of multiple myeloma; diagnosis and management of associated disorders; accelerating diagnostic evaluation (differential diagnosis); facilitating appropriate timing, etc.; and systemic therapy.

[0056] For practical clinical laboratory analysis in serum or urine, such testing should be: sensitive; rapid; dependable; and should be able to detect M protein. Such testing may also utilize specific assays to identify the protein according to its heavy chain and light chain type. The laboratory method may comprise one of the following: use of cellulose acetate to produce a localized band; use of agarose gel electrophoresis to produce a localized band; immunofixation (IF) to confirm the presence of an M band; and immunoelectrophoresis (IE)- to determine the
immunoglobulin heavy chain class. An example might include monoclonal patterning of serum M protein by densitometry after alactophoresis on agarose gel. Another example might include immunofixation of serum with antisera to IgG, IgA, IgM kappa (κ) and lambda (λ) for band localization. Another example might include immunofixation of concentrated urine with antisera to κ and λ light chains.

[0057] The presently described chromatographic test may be used adjunctively where the following indications, without limitation, may be present: suspect multiple myeloma (MM); suspect Waldenstrom’s macroglobulinemia (WM); suspect primary systemic amyloidosis (AL), or upon presentation of additional conditions, such as unexplained weakness and fatigue; elevation of erythrocyte sedimentation rate (ESR); anemia; unexplained back pain; osteoporosis; osteolytic lesions or fractures; hypercalcemia; hance-jones proteinuria; renal insufficiency; recurrent infections; unexplained sensorimotor peripheral neuropathy; carpal tunnel syndrome; refractory congestive heart failure; nephrotic syndrome; orthostatic hypotension; malabsorption; weight loss; change in tongue or voice; paresthesias; numbness; increased bruising, bleeding; or steatorrhea. It should be noted that Negative (SEP) requires immunofixation in MM; and AL or related disorders are suspected clinically. Quantitation of Immunoglobulin-Nephelometry, particularly with regard to immunofixation of urine may be called for with nephrotic syndromes of unknown cause, AL, and light-chain deposition diseases.

[0058] Clinical applications of free light chain immunoassays such as the tests described herein include application to the following most common light chain diseases, among others: multiple myeloma; Waldenstrom’s macroglobulinemia; (AL) Amyloidosis, and Fanconi’s Syndrome. The prevalence of these light chain diseases provides good evidence of usability of the test herein for screening and adjunctive testing. With regard to multiple myeloma, approximately 45,000 Americans currently have myeloma, and the American Cancer Society estimates that approximately 14,600 new cases of myeloma are diagnosed each year in the United States. With regard to Waldenstrom’s macroglobulinemia, the median age of development is 60 years old, with cases being reported between the ages of 30-90. The disease occurs in 5 out of 100,000 people over 50 years old, with men comprising 2/3 of cases reported.
each year. With regard to AL amyloidosis, typically, AL amyloidosis affects people in their 50’s and 60’s, but people in their 20’s and 30’s have been diagnosed as well. Amyloidosis is a rare disease, with an estimated prevalence of 1 per 60,000. With regard to Fanconi’s Syndrome, Fanconi’s syndrome is an impairment in proximal tubular function of the kidney. This impairment causes certain compounds, which should be absorbed back into the bloodstream by the kidneys, to be excreted in the urine instead. Fanconi’s syndrome can be genetic or acquired later in life, and this disorder affects 1 out of 350,000 people. Table 3, below further evidences the significance of each disease state (monitoring):
### TABLE 3: SIGNIFICANCE OF DISEASE STATES (MONITORING)

<table>
<thead>
<tr>
<th>Monoclonal Gammopathies (MG):</th>
<th>Avg. Yrs</th>
<th>USA</th>
<th>CANADA</th>
<th>EUR- OPE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subcategories:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGUS</td>
<td>15</td>
<td>*25,000</td>
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<tr>
<td>MM</td>
<td>7</td>
<td>54,721</td>
<td>1,850</td>
<td>54,720</td>
</tr>
<tr>
<td>WM</td>
<td>10</td>
<td>54,370</td>
<td>not available</td>
<td>Not available</td>
</tr>
<tr>
<td>AL</td>
<td>10</td>
<td>7,880</td>
<td>not available</td>
<td>Not available</td>
</tr>
<tr>
<td>FS</td>
<td>10</td>
<td>6,740</td>
<td>not available</td>
<td>Not available</td>
</tr>
<tr>
<td>RI/RF (due to MG)</td>
<td>10</td>
<td>41,041</td>
<td>4,300</td>
<td>222,972</td>
</tr>
<tr>
<td>RI/RF (due to Diabetes)</td>
<td>15</td>
<td>300,000</td>
<td>not available</td>
<td>Not available</td>
</tr>
<tr>
<td>RI/RF (due to Hypertension)</td>
<td>15</td>
<td>91,683</td>
<td>not available</td>
<td>Not available</td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td></td>
<td><strong>581,435</strong></td>
<td><strong>6,150</strong></td>
<td><strong>277,692</strong></td>
</tr>
<tr>
<td><strong>New Cancer Cases/Year:</strong></td>
<td></td>
<td>approx. 1.37 million</td>
<td>approx. 145,500</td>
<td>Approx. 6 million</td>
</tr>
<tr>
<td><strong>DIABETES market</strong></td>
<td></td>
<td>approx. 27 million</td>
<td>approx. 2.8 million</td>
<td>approx. 98 million</td>
</tr>
</tbody>
</table>

*MGUS 25,000 is an estimation of numbers from five Centers of Excellence; Statistics from American Cancer Society, National Diabetes Statistics, Canadian Cancer Society, Diabetes in Canada, International Diabetes Federation, World Health Organization (GLOBOCAN 2000)
[0059] Table 4 shows an expanded model relevant to monitoring of monoclonal gammopathies:

**TABLE 4: MONOCLONAL GAMMOPATHIES**

<table>
<thead>
<tr>
<th>Monoclonal Gammopathies</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of cases per year</td>
</tr>
<tr>
<td><strong>United States</strong></td>
<td></td>
</tr>
<tr>
<td>MGUS</td>
<td>25,000</td>
</tr>
<tr>
<td>MM</td>
<td>54,721</td>
</tr>
<tr>
<td>WM</td>
<td>54,370</td>
</tr>
<tr>
<td>AL</td>
<td>7,880</td>
</tr>
<tr>
<td>FS</td>
<td>6,740</td>
</tr>
<tr>
<td>RI/RF</td>
<td>41,041</td>
</tr>
<tr>
<td>RI/RF - due to Diabetes</td>
<td>300,000</td>
</tr>
<tr>
<td>RI/RF – due to</td>
<td>91,683</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>581,435</td>
</tr>
</tbody>
</table>

| **Canada**              |             |                      |         |
| MGUS                    | 7           |                      |         |
| MM                      | 1,850       | 10                   | 12,9500 |
| WM                      | 10          |                      |         |
| AL                      | 10          |                      |         |
| FS                      | 10          |                      |         |
| RI/RF                   | 4,300       | 10                   | 30,100  |
| *RI/RF - due to Diabetes| 15          |                      |         |
| *RI/RF – due to         | 15          |                      |         |
| Hypertension            |             |                      |         |
| **Total:**              |             |                      | 43,050  |

<p>| <strong>Europe</strong>              |             |                      |         |
| MGUS                    | 7           |                      |         |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>54,720</td>
<td>10</td>
<td>383,040</td>
</tr>
<tr>
<td>WM</td>
<td>10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FS</td>
<td>10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>RJ/RF</td>
<td>222,972</td>
<td>10</td>
<td>1,560,804</td>
</tr>
<tr>
<td>*RJ/RF - due to Diabetes</td>
<td>15</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>*RJ/RF - due to Hypertension</td>
<td>15</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>277,692</td>
<td>1,943,844</td>
<td></td>
</tr>
</tbody>
</table>

**Totals**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MGUS</td>
<td>25,000</td>
<td>7</td>
<td>175,000</td>
</tr>
<tr>
<td>MM</td>
<td>111,291</td>
<td>10</td>
<td>779,037</td>
</tr>
<tr>
<td>WM</td>
<td>54,370</td>
<td>10</td>
<td>380,590</td>
</tr>
<tr>
<td>AL</td>
<td>7,880</td>
<td>10</td>
<td>55,160</td>
</tr>
<tr>
<td>FS</td>
<td>6,740</td>
<td>10</td>
<td>47,180</td>
</tr>
<tr>
<td>RJ/RF</td>
<td>268,313</td>
<td>10</td>
<td>1,878,191</td>
</tr>
<tr>
<td>RJ/RF - due to Diabetes</td>
<td>300,000</td>
<td>15</td>
<td>2,100,000</td>
</tr>
<tr>
<td>RJ/RF - due to Hypertension</td>
<td>91,683</td>
<td>15</td>
<td>641,781</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>865,277</td>
<td>6,056,939</td>
<td></td>
</tr>
</tbody>
</table>

[0060] Not only does the presently described test permit front-line monitoring (as well as further confirmatory testing/adjunctive testing) of candidates that fall under the monoclonal gammopathies heading, but the present test also allows for front line screening of patients. Table 5, below indicates the relevant screening population in the United States (as exemplary of the need for such front line screening):
## TABLE 5: RELEVANT SCREENING POPULATION IN THE USA AND SCREENING MODELS

<table>
<thead>
<tr>
<th>Age &amp; Sex in USA</th>
<th>*Pop/Ages *75% Screened for PSA &amp; *81.4% Screened for PAP</th>
<th>*50% Estimated to be screened for MG</th>
<th>Test</th>
<th>DiaSys Projected Annual Sales</th>
<th>Price $</th>
</tr>
</thead>
<tbody>
<tr>
<td>30- Male</td>
<td>20,630,000 <strong>not</strong></td>
<td>10,315,000</td>
<td>$15.00</td>
<td>$154,7</td>
<td>$</td>
</tr>
<tr>
<td>40 Female</td>
<td>20,977,000 available</td>
<td>16,991,370</td>
<td>8,495,685</td>
<td>$15.00</td>
<td>25,000</td>
</tr>
<tr>
<td>40- Male</td>
<td>36,190,000 27,142,500</td>
<td>13,571,250</td>
<td>$15.00</td>
<td>$203,5</td>
<td></td>
</tr>
<tr>
<td>60 Female</td>
<td>37,716,000 30,549,960</td>
<td>15,274,980</td>
<td>$15.00</td>
<td>68,750</td>
<td>$229,1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24,700</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>53,725</td>
</tr>
</tbody>
</table>

*population based on the US Government census 2001, *PSA information taken from JAMA. 2003 Mar 19;289 (11): 1414-20, *Use of Pap smears for women 18 years of age and over according to selected characteristics: United States, selected years 1987-2000; Center for Disease Control and Prevention: Health, United States 2003. *50% of PSA/PAP screening population figures **due to unavailable data on males in the 30-40 age group screened for PSA in the USA, MG screening was estimated using 50% of the total male population in this age group

### Screening Models:

<table>
<thead>
<tr>
<th>Screening Programs</th>
<th>% Screened</th>
<th>Ages Screened (Projection for MG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap Smears</td>
<td>*81.4</td>
<td>Ages 18-64</td>
</tr>
<tr>
<td>PSA (Prostate)</td>
<td>*75</td>
<td>Ages 50 and above</td>
</tr>
</tbody>
</table>

*Percentages from the Centers for Disease Control and Prevention; and JAMA (listed above)
[0061] The presently described chromatographic immunoassay overcomes the problems described generally in the Background, and specifically with reference to Table 2, therein by providing a product with one or more of the Below described features and benefits. In one embodiment, a chromatographic immunoassay based on lateral flow technology can detect urine free light chains at <10 mg/L. Additionally, an assay may be provided specific for free and bound light chains and able to detect light chains complexed to heavy chains or polymerized to other light chain units. Further, a monoclonal cocktail nature of the capture antibody may allow for multi-epitope targeting. Unconcentrated urine sample is assayed; and both free and bound light chains bind to migratable conjugated monoclonal antibody cocktail. If light chains are present, an antigen-conjugate complex is formed, the sample will migrate to membrane bound test zone of free light chains-the complex will not bind there, and the absence of clear line in the test zone indicated the presence of light chains at <10 mg/L. The assay has no cross-reactivity to other urine constituents including pH, glucose, ketones, osmolarity, albumin and organic acids. There is also no cross-reactivity between light chains. The specimen does not require pre-treatment. Results read in 5 minutes. Sensitivity is better than about 88% (approx 10 mg/L urinary free light chain), and specificity is better than about 99%.

[0062] One embodiment of the present test comprises an in-vitro assay for bence-jones proteins, comprising a unique dipstick format, kappa and lambda separate detection, qualitative determination, minimal operator time, reduced processing time, antibody linked specificity, high sensitivity, and an all reagents packaging wherein everything is supplied/there is nothing else to buy.

[0063] The test provides for a minimum required sample. In one embodiment, such sample may be as little as 0.3 ml of non pre-treated, un-concentrated urine. In another embodiment, a test kit includes reaction devices for both kappa and for lambda (e.g., blue for kappa, white for lambda). Exemplary advantages of the present testing methods and devices are illustrated by Table 6, below:
**TABLE 6: ADVANTAGES OF THE PRESENT TEST OVER CURRENT MEDICAL STANDARDS FOR URINE LIGHT CHAIN EVALUATION**

<table>
<thead>
<tr>
<th>Test</th>
<th>Requirements</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Protein Electrophoresis</td>
<td>Concentrated specimen</td>
<td>Sensitive in concentrated urine (10 mg/L); May scan to obtain quantitative results</td>
<td></td>
<td>Subjective interpretation; Protein may be lost with concentration; False bands can appear from concentration; Problems with specimen with heavy proteimuria and 24-hr collections</td>
</tr>
<tr>
<td>Immunofixation Electrophoresis on urine</td>
<td>Concentrated Specimen</td>
<td>Sensitive for concentrated urine (2-5 mg/L)</td>
<td></td>
<td>Non-quantitative; Expensive antisera; Slow; Technically difficult</td>
</tr>
<tr>
<td>The presently described chromatographic test</td>
<td>No Pre-treatment</td>
<td>88% (10 mg/L)</td>
<td>&gt;99%</td>
<td></td>
</tr>
</tbody>
</table>

[0064] In one embodiment, the present device comprises a chromatographic immunoassay for detection of free light chains in non-centrifuged or un-concentrated/un-treated urine. The chromatographic immunoassay may comprise a tube (which may be used in clinical settings, e.g., a doctors office or laboratory) with a dipstick, or a cassette (which may be used, e.g., in homes or point-of-care settings) with a lateral flow pad. The pad may be a conjugate, multi-parameter pad or a single pad.

[0065] In another embodiment, a rapid chromatographic test is provided for detection of free light chains in urine, wherein the rapid test produces results within 5 minutes, with a sensitivity greater than about 88% (for approximately 10mg/L urinary free light chain) and a specificity of greater than about 99%.
[0066] In another embodiment, a rapid chromatographic test is presented for detection of free light chains in urine, including a reaction device for kappa, and a reaction device for lambda.

[0067] In another embodiment, the present disclosure presents a method of diagnosing an indicator for a medical condition precipitating free light chains in urine, the method comprising exposing a chromatographic test device to untreated/unspun urine, wherein the chromatographic test device indicates presence of said free light chains in the urine. The medical condition may be one or more of: Multiple Myeloma, micromolecular myeloma, Waldstrom’s Macroglobulinemia, Chrmic Lymphatic Leukemia, Amyloidosis, lupus erythematosus, Monoclonal Gammopathies of Undetermined Significance, Faconi’s Syndrome, Renal Insufficiency/Renal Failure, Renal Failure due to Diabetes, Renal failure due to hypertension, and acute rheumatoid arthritis. The free light chain may be one or more of a monoclonal free light chain and a polyclonal free light chain.

[0068] In another embodiment, the present disclosure provides a cartridge, comprising at least one lateral strip pathway. In such cartridge, the lateral flow may begin at one origin (sample application) and proceed laterally along at least two distinct pathways.

[0069] In another embodiment, a home test cartridge is provided, including a conjugate pad or a lateral flow dipstick.

[0070] Immunoglobulin molecules normally consist of pairs of polypeptide chains of unequal size bound together by several disulphide bridges. In each immunoglobulin molecule there are a pair of heavy chains, which may be either gamma, alpha, mu, delta or epsilon type, and a pair of light chains which may be either kappa or lambda. In some pathological conditions such as multiple myeloma, there is a proliferation of one antibody-producing plasma cell leading to excess production of light chains of one specific kind. These free monoclonal light chains can be found in urine and plasma and are known as Bence-Jones Proteins (BJP). The present test provides improved chromatographic assays for the detection of Bence Jones proteins in urine.
Chromatographic Test Strips

[0071] Chromatographic test strips are utilized for the detection of an analyte of interest in a sample. While the analyte of interest is free light chains (kappa and/or lambda), one exemplary embodiment provides a control zone capable of detecting or binding to a large class of immunochemicals, including proteins and antibodies. The detection of the presence of an analyte of interest in the detection zone may indicate the presence or absence of disease. The detection of the presence of an analyte in the control zone may also indicate that the test strip is working.

[0072] As noted above, chromatographic test strips are used to detect Bence Jones proteins in untreated urine. Exemplary test strips utilize a dry porous carrier material, such as a strip of nitrocellulose. If desired, the nitrocellulose can be backed with moisture impermeable material, such as polyester sheet. This backing may be transparent to facilitate a densitometric analysis, or transmission of light for quantitation of total Bence Jones protein within the porous carrier detection zone. Using nitrocellulose as the porous carrier material has considerable advantage over more conventional strip materials, such as paper, because nitrocellulose has a natural ability to bind proteins without requiring prior sensitization. Specific binding reagents, such as immunoglobulins, can be applied directly to nitrocellulose and immobilized thereon. No chemical treatment is required which might interfere with the essential specific binding activity of the reagent. Moreover, nitrocellulose is readily available in a range of pore sizes, which facilitates the selection of a carrier material to suit particularly requirements such as sample flow rate. In one exemplary embodiment, the nitrocellulose has a pore size of at least one micron. In another exemplary embodiment, the nitrocellulose has a pore size not greater than about 20 microns.

[0073] In an exemplary embodiment, the labeled specific binding reagent comprises a specific binding reagent attached to a particulate label. Such "direct labels", e.g. gold sols, and non-metallic colloids, are already known per se. They can be used to produce an instant analytical result without the need to add further reagents in order to develop a detectable signal. They are robust and stable and can therefore be used readily in an analytical device that is stored
in the dry state. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes. In one embodiment, the particulate label is a gold sol, which can be readily visible to the eye if it becomes bound in the detection zone. If desired, the assay result can be read instrumentally, e.g. by color reflectance, or scanned into a computer to measure band intensity. The reaction zone and control zone may be viewed manually or by an optical/electronic reader. The purpose of a reader is to allow consistency and quantification of the reading. Readers in accordance with the present invention include scanner, image analysis, or a camera, such as a digital camera. A less expensive way to read the test results is to use a document scanner such as an HP scanner. This type of scanner connects to a PC via the printer port or the USB port. The assays of the present invention may be placed upside down on the reader and scanned. The result is an image file that can be viewed on the PC and analyzed by image analysis software. Another reader of the present invention runs an application program (run on a PC) that would control the image capture and presentation of data to the user. In an application program, results can be stored in a PC and used to evaluate the progress of the disease and/or treatment if the test is repeated periodically. A third reader would be a digital camera. The camera would be positioned over the test and control areas of the present invention. Digital images can be stored and retrieved at a later date to monitor disease or treatment thereof.

[0074] In an exemplary embodiment, a chromogenic mobile specific binding partner, or mobilizable labeled specific binding reagent is applied to a conjugate pad. Conjugate pads according to the present invention may be made from materials, which include glass fibers, paper, or polypropylene filters. In one embodiment, the conjugate pad is manufactured from glass fibers. The conjugate pad is presoaked in solution containing at least one mobile specific binding partner, which is specifically reactive with analytes of interest, for example lambda, or kappa monoclonal free light chains.

[0075] When the labeled specific binding reagent is impregnated and dried into conjugate pad, the test strips are optimized for competitive binding type assays. According to such methods, an immobilized specific binding reagent is selected to competitively bind with labeled binding reagent and analyte of interest. Likewise, the labeled specific binding reagent is
preselected to be a specific binding analogue of the analyte that will bind competitively with the immobilized specific binding reagent located in the porous membrane matrix. The mobile specific binding partner is preselected from anti-free kappa and/or anti-free lambda antibodies conjugated with a visible colloidal metal. Although other colloidal metals that one of skill in the art would use to bind to an antibody to make a visible color may work, gold provides an easily discernable red band in relation to the presence of analyte. In one embodiment, the mobile specific binding partner may be one conjugated polyclonal antibody. In another embodiment, the mobile specific binding partner may be one conjugated monoclonal antibody or a conjugated monoclonal antibody cocktail.

[0076] By incorporating a mobile specific labeled reagent in a separate conjugate pad, rather than pre-dosed onto the carrier material that also incorporates the detection zone, the following advantages can be obtained: (1) ease of manufacture, because Bence-Jones Protein assays require the analysis of different classes of free light chains, i.e. both the kappa and the lambda type, the incorporation of the labeled reagent specific to each in separate conjugate pads avoids the need to apply the labeled reagent in a special zone in the carrier, which may need careful pre-treatment; (2) uniformity in test strip assembly, because the remaining components in the test strip have similar dimensions, they can be easily manufactured and assembled into test strips; and (3) enhancing sensitivity of the test because a substantial quantity of the liquid sample is able to take up the chromogenic mobile labeled specific binding reagent before migrating through the carrier material to the detection zone, enhancing potential reaction time without significantly increasing overall test time.

[0077] In an exemplary embodiment, the assay device identifies one analyte per test strip. This is done by having one labeled binding reagent in one conjugate pad, and one immobilized specific binding reagent in the porous carrier. Multiple test strips may be run side by side that are manufactured to identify the presence of various analytes of interest. In another embodiment, a kappa sensing test strip is run next to a lambda sensing test strip. Side by side analysis allows for a reduced risk of contamination and is easier to use than state of the art test kits. The two strips
also need not include separate sample application regions, but instead may share a sample well. In such case, sample flows from the sample well down diverging flow paths.

[0078] In another embodiment, the assay device identifies more than one analyte (e.g. kappa and lambda monoclonal free light chains) in a single sample by a competitive binding type assay. This is done utilizing a device capable of running two or more test strips simultaneously, in a generally side-by-side fashion. Test strip one contains a first conjugate pad having a first labeled specific binding reagent impregnated and dried into the conjugate pad. Test strip one also has a first immobilized specific binding reagent in the dry porous carrier pre-selected to specifically bind with the first labeled specific binding reagent. The first labeled specific binding reagent, however, is preselected to specifically bind with analyte that will bind competitively with the first immobilized specific binding factor. The first labeled specific binding factor will bind to monoclonal free lambda light chains. Accordingly when a sample containing monoclonal free lambda light chains is added to test strip one, the lambda free light chains in the sample will bind with the first labeled specific binding reagent upon contact and form an analyte/labeled binding reagent complex. This complex formation decreasing the ability of first labeled specific binding reagent to bind with first immobilized specific binding partner. Although not wishing to be bound by any theory, it is believed that in a positive sample containing monoclonal free lambda light chains, the epitopes or binding areas on the first labeled specific binding reagent become saturated with analyte, and lose the ability to also bind with first immobilized specific binding reagent upon contact. Accordingly, in a negative sample, first labeled specific binding reagent does not bind to analyte in sample, and is free to bind with the first immobilized specific binding reagent upon contact therewith. Test strip two contains a second conjugate pad having a second labeled specific binding reagent impregnated and dried into the second conjugate pad. Test strip two also has a second immobilized specific binding reagent in the dry porous carrier selected to specifically bind with the analyte of interest such as free kappa light chains. The second labeled specific binding reagent, however, is preselected to specifically bind with analyte that will bind competitively with the second immobilized specific binding factor. This second labeled specific binding factor will bind to monoclonal free kappa light chains. Accordingly when a sample containing monoclonal free kappa light chains is added to test strip two, the
kappa free light chains in sample will bind with the second labeled specific binding reagent upon contact to form a analyte/labeled binding reagent complex. Complex formation decreasing the ability of second labeled specific binding reagent to bind with second immobilized specific binding reagent. Although not wishing to be bound by any theory, it is believed that in a positive sample containing monoclonal free kappa light chains, the epitopes or binding areas on the second labeled specific binding reagent become saturated with analyte, and lose the ability to also bind with second immobilized specific binding reagent upon contact. Accordingly, in a negative sample, second labeled specific binding reagent does not bind to analyte in sample, and is free to bind with the second immobilized specific binding reagent upon contact therewith.

[0079] In alternative embodiments, conjugate pad can incorporate several labeled mobile specific binding reagents to indicate the presence of multiple analytes in a single sample. For example, both kappa and lambda monoclonal free light chains can be detected. This will facilitate the manufacture of a multiple analyte testing devices for analytes of interest, e.g. kappa and lambda detection in a single test strip. In this embodiment, immobilized specific binding reagents are striped at various locations along the porous carrier to create multiple detection zones. For example, it a first immobilized specific binding reagent may be striped along the porous carrier at a position closer to the conjugate pad than a second immobilized specific binding reagent. In this case, the first immobilized specific binding reagent is said to be upstream from the second immobilized specific binding reagent. Conversely, the second immobilized specific binding reagent is said to be downstream of the first immobilized specific binding reagent.

[0080] The conjugate pad may be in direct moisture-conductive contact with the porous material, and the detection zone on the porous carrier material is spaced away from the region of contact between the porous carrier material and the conjugate pad. In such an embodiment, the quantity of liquid sample required to saturate the conjugate pad is preferably not less than the quantity of liquid sample capable of being absorbed by the mass of porous carrier material linking the conjugate pad and the detection zone. In other words, the liquid capacity of the conjugate pad is at least equal to the liquid capacity of the working portion of the porous carrier.
The conjugate pad is optimized to hold approximately between 100 to 300 μl of liquid sample, preferably about 200 μl.

[0081] In one exemplary embodiment, the test strip may further comprise an absorbent pad. Absorbent pad is μ laced at the far end of the immunochromatographic strip. The major advantage of using an absorbent pad is that the total volume of sample that enters the test can be increased. This increased volume can be used to wash away unbound detector reagent from the nitrocellulose membrane. The net result is that the assay readout zone will have a lower background and assay sensitivity can be enhanced. Since the volume of sample that ultimately contributes to signal is controlled by the volume required to solubilize the detector reagent, and not by the total volume of sample that enters the device, the addition of the absorbent pad may not have a dramatic impact on overall assay sensitivity.

[0082] In the exemplary embodiment, the labeled reagent is a specific binding partner for the analyte. The labeled reagent, the analyte (if present) and the immobilized unlabelled specific binding reagent compete together in a "competition" reaction. This results in the labeled reagent being bound in the detection zone if analyte is not present in the sample. The labeled specific binding partner is selected to be a specific binding analogue of the analyte that will bind competitively with the immobilized specific binding reagent located in the porous membrane matrix.

Testing Cassette

[0083] In a further embodiment, the porous carrier is linked via the conjugate pad to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous carrier. The porous carrier and the conjugate pad may contained within a moisture-impermeable casing or housing and the porous receiving member can be placed below a gap in the housing or front plate and can act as a means for permitting a liquid sample to enter the housing and reach the porous carrier. Sample receiving member can be made from any porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be unidirectional (i.e. with pores running wholly or predominantly parallel to an
axis of the member) or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Porous sample receiving members can be made from paper or other cellulosic materials, such as nitrocellulose. The material comprising the porous receiving member may be chosen such that the porous member can be saturated with aqueous liquid within a matter of seconds. The liquid must thereafter permeate freely from the porous sample receiving member into the conjugate pad. Typically the porous receiving member is located under the gap in the housing where sample is first added to the test strip or system. The porous receiving member is the first area of the test strip to contact liquid sample. The porous receiving member is in contact with the conjugate pad such that sample can flow between the two pads. The conjugate pad is disposed upon the porous dry carrier so that liquid can flow between that conjugate pad and the porous dry carrier.

[0084] In an exemplary embodiment, the housing, which may be provided with means, e.g., appropriately placed aperture or apertures, which enable the detection zone of the porous solid phase carrier material (carrying the immobilized unlabelled specific binding reagent) to be observable from outside the housing so that the result of the assay can be observed. The housing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with at least one gap above the receiving member. The housing may be constructed to have a transparent back late to facilitate a densitometric analysis, or transmission of light through the test strip to facilitate quantitation of total Bence Jones proteins. If desired, the housing may also be provided with further means which enable a further zone of the porous solid phase carrier material to be observed from outside the housing and which further zone incorporates one or more control reagents which enable an indication to be given as to whether the assay procedure has been completed. The housing provides at least one well capable of holding at least one test strip of the present invention. The housing may have at least two wells capable of holding a test strip each, so that two test strips can be run side-by-side within the same housing.

[0085] In such embodiment, the housing performs a critical task of ensuring contact among the system components. The test strips of the embodiment typically comprise a receiving
member, a conjugate pad, a porous carrier and an absorbent pad. It is important that these components be in sequential fluid communication. This means that when liquid sample is applied to the system, liquid is able to travel by capillarity between various system components because they are in contact. The housing places pressure in the system where the various test strip components overlap. If the pressure is too high, the components can be crushed, which can block pores and the ability of liquid sample to flow. A crushed conjugate pad may prevent contact between positive sample and system components such as porous carrier and absorbent pad. This may contribute to false positive results. A crushed porous member may also prevent the movement of analyte or labeled binding reagent throughout the porous member further causing inaccurate results. Conversely, if the pressure in the system is too low, the system may lose sequential fluid communication. In the preferred embodiment, the housing provides optimum pressure to ensure flowability of liquid sample throughout the overlapping components in the test strip. It is understood in the art, that flowability of liquid sample, analyte, and complexes throughout system components can be adjusted by trial and error. One of skill in the art may adjust flowability by altering porosity of each component, size of each component, dimensions of each component, including thickness of each component, as well as pressure on system components.

[0086] In an exemplary embodiment, the housing comprises a hollow elongated casing containing at least two wells. Each well is capable of supporting a test strip having a dry porous nitrocellulose carrier, which communicates indirectly with the exterior of the casing via a gap and a receiving member. The porous nitrocellulose carrier and the sample receiving member being linked via a conjugate pad such that any sample reaching the porous carrier must first pass through the conjugate pad, and the sample receiving member. The conjugate pad and sample-receiving member when taken together act as a reservoir from which urine is released into the porous carrier. The conjugate pad containing a highly specific anti-Bence Jones antibody bearing a colored "direct" label, the labeled antibody being freely mobile within the conjugate pad and the porous carrier when in the moist state. The porous carrier containing a detection zone on the carrier spatially distant from the conjugate pad and a highly-specific unlabelled monoclonal free light chain protein which is permanently immobilized on the carrier material and is therefore not
mobile in the moist state. The monoclonal free light chain protein is preselected from either monoclonal free lambda, or kappa light chains.

[0087] In exemplary embodiments, the porous dry carrier further comprises a control zone designed to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with protein that will bind to the labeled reagent, e.g. Protein A, to confirm that the sample has permeated the test strip. As the purpose of the control zone is to indicate to the user that the test has been completed, the control zone should be located downstream from the detection zone in which the desired test result is recorded. A positive control indicator therefore tells the user that the sample has permeated the required distance through the test device.

[0088] In exemplary embodiments, labeled specific binding reagent comprises a chromogenic label. The label can be any entity the presence of which can be readily detected. The label may be a direct label, i.e., an entity which, in its natural state, is readily visible either to the naked eye, or with the aid of an optical filter, reader, and/or applied stimulation, e.g. UV light to promote fluorescence. For example, minute colored particles, such as metallic sols (e.g. gold), are very suitable. Concentration of the label into a small zone or volume should give rise to a readily detectable signal, e.g. a strongly colored area. This can be evaluated by eye, or by instruments if desired. In the competitive reaction, a signal indicates the absence of analyte, because the labeled binding reagent has not bound to the analyte of interest and is free to bind to the immobilized specific binding reagent located in the reaction zone. Conversely, the lower the signal, the more analyte in the sample, because the labeled binding reagent has bound to the analyte of interest and cannot also bind to the immobilized specific binding reagent located in the reaction zone. Accordingly, the presence or intensity of the signal from the label that becomes bound in the detection zone can provide a qualitative or quantitative measurement of analyte in the sample.

[0089] In all embodiments, it is essential that the labeled reagent and analyte complexes thereof, and analyte (if any), migrate with the liquid sample as it progresses to the detection zone. Preferably, the flow of sample continues beyond the detection zone and sufficient sample
is applied to the porous carrier material in order that this may occur and that any excess labeled reagent which does not participate in any binding reaction in the detection zone is flushed away from the detection zone by this continuing flow. An absorbent pad can be provided at the distal end of the carrier material. The absorbent sink may comprise, for example, Whatman 3 MM chromatography paper, and should provide sufficient absorptive capacity to allow any unbound conjugate to wash out of the detection zone.

[0090] The immobilized reagent in the detection zone is preferably a free light chain protein. A free light chain protein capable of binding competitively to labeled binding reagent when similar monoclonal free light chain protein may be present in a sample. The monoclonal free light chain is extracted and purified using techniques known in the art of extracting and purifying proteins. The free light chain is typically stripped along the narrow width of the porous carrier material. The free light chain may be stripped along the dry porous carrier to create a detection zone approximately 1.0 mm in width. When a solution of free light chain is stripped along dry porous carrier it becomes permanently immobilized therein. The porous carrier material may be in the form of a strip or sheet to which during manufacture of the device, one or more reagents can be similarly applied in distinct zones. During use, the liquid sample is allowed to permeate through the sheet or strip from one side or end to another.

[0091] In an exemplary embodiment, multiple test strips are run side by side, having separate conjugate pads impregnated with at least one different chromogenic mobile specific binding reagents for binding to multiple analytes of interest such as kappa and lambda monoclonal free light chains. Two test strips may be placed side by side in the housing. The first test strip has a first conjugate pad, containing a first mobile specific binding reagent. The first mobile specific binding reagent is chromogenic and capable of binding to lambda light chains. The second test strip comprises a second conjugate pad comprising a second chromogenic mobile specific binding reagent. The second mobile specific binding reagent is chromogenic and capable of binding to kappa light chains.

[0092] When multiple test strips are run side by side, multiple reaction sites may be included to detect multiple analytes of interest. There may be two test strips placed side by side
in the housing where each strip contains two reaction zones approximately 1.0 mm in width. The first test strip has a first reaction zone or site and a second reaction zone or site. The first reaction site comprises a first immobilized specific binding reagent capable of immobilizing the labeled binding reagent from the conjugate pad in relation to the absence of analyte in the sample. The second reaction zone may be a control zone designed merely to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with an protein that will bind to the mobile labeled binding reagent, e.g. Protein A, to confirm that the sample has permeated the test strip.

[0093] The second test strip has a third reaction zone and a fourth reaction site. The third reaction site comprises a second immobilized specific binding reagent capable of immobilizing the mobile specific binding reagent from the second conjugate pad in relation to the absence of analyte in the sample. The fourth reaction zone is a control zone designed merely to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with an protein that will bind to the labeled reagent, e.g. Protein A, to confirm that the sample has permeated the test strip. The designation of "first", "second", "third" and "fourth" reaction zone is not meant to limit the present invention in any way. The designation is meant to show that there are multiple tests strips of substantially similar design operating in unison, each test strip preferably having a reaction zone and a control site. For example, if three test strips were run simultaneously, the reaction zones would be designated and the fifth and sixth reaction zones because two zones are added with the addition of an additional strip.

[0094] In exemplary embodiments, porous dry carrier has a first end at which chromatographic urine transport begins and a second end at which chromatographic solvent transport ends. The length of porous dry carrier comprises a first reaction site, and a second reaction site. Second reaction site acts as a control that indicates that sample has been transported throughout the length of test strip. All parameters on the porous dry carrier or cellulosic membrane may be changed but the width will not affect the sensitivity (provided it is above about 4 mm, where edge drag effects come in to play). Absorbent pad is placed upon cellulosic membrane opposite conjugate pad such that capillary action can draw sample from first end to
second end thereby transporting sample through reaction site, and second reaction site. Absorbent pad can be fabricated from any material that has a propensity to wick liquid such as a sponge or paper towel material and is typically 17 mm long and 6 mm wide. The size and thickness must be such that it provides enough capillarity to transport sample from first end to second end.

[0095] The first reaction site is impregnated with a first immobilized specific binding reagent capable of reacting with and the immobilization of the mobile labeled binding reagent when analyte such as free light chain is absent in sample. In test strips of the present invention the immobilized specific binding reagent is free kappa or free lambda light chain proteins. In the presence of free light chains in a sample, the immobilized free light chains bind competitively with the labeled binding reagent, and to the free kappa or lambda light chain proteins in the sample. The second reaction site, or control site is downstream of the first reaction site and is impregnated with a second immobilizing specific binding factor. Typically a chemical capable of specifically binding to immunochemicals, such as Protein A. Although not wishing to be bound by any theory, Protein A is capable of effectively collecting and immobilizing antigen-antibody complexes by binding to the Fc section of immunoglobulins. Control reaction site acts as a positive control and is relied upon to indicate that capillary action has carried test sample, mobile specific binding partners, and analyte/chromogenic mobile specific binding partner complexes thereof throughout the length of test strip.

[0096] The invention also provides an analytical method in which a device as set forth above is contacted with an aqueous liquid sample suspected of containing the analyte, such that the sample permeates by capillary action via the conjugate pad through the porous solid carrier into the detection zone and the labeled reagent migrates therewith to the detection zone, the presence of analyte in the sample being determined by observing the extent (if any) to which the labeled reagent passes through the detection zone, and does not bind thereto. Accordingly, the absence of analyte in the sample being determined by observing the extent (if any) to which the labeled reagent becomes bound in the detection zone. In such methods, liquid sample suspected of containing analyte is first obtained. Preferably the liquid sample is urine because Bence Jones
proteins are often small enough to pass through the glomerular filter and pass with urine from the body. Sample is obtained by collecting urine midstream. Next, aliquots of urine are portioned into between 100 to 300 microliters aliquots using a syringe or pipette, preferably 200 microliters. Next liquid sample is injected into the device using the syringe or pipette. In one embodiment, 200 microliters of sample is injected upon the first receiving member located below the first gap. In order to apply sample to the second receiving member, the aliquoting steps are repeated and sample is applied to second receiving member. It is not necessary to change the syringe or pipette between applications to the receiving members because the same sample is applied to the separate receiving members.

[0097] After applying sample to the device, sample permeates laterally throughout the device by capillary action via the conjugate pad through the porous solid carrier into the detection zone and the labeled reagent migrates therewith to the detection zone, the presence of analyte in the sample being determined by observing the extent (if any) to which the labeled reagent passes through the detection zone, and does not bind thereto. This is because in a positive sample, analyte of interest will bind to labeled binding reagent to form an analyte/labeled binding reagent complex. Such complexes have a decreased ability to bind to the immobilized reagent in the porous carrier. It has been surprising found that a lateral flow device of the present invention provides quick, and accurate indication for the presence of free light chains, and classes of free light chains.

[0098] By way of example only, some embodiments will now be described in detail with reference to the accompanying drawings.

[0099] Referring to FIG. 1, the device comprises a test strip (1). Test strip (1) is preferably 60 mm. in length, and 6 mm. in width. Test strip (1) is largely comprised of a dry porous carrier (2) having a first end (3) at which chromatographic transport begins and a second end (4) at which chromatographic solvent transport ends. The length of dry porous carrier (2) comprises a first reaction site, or detection zone (5), and a second reaction site or control zone (6). Second reaction site (6) acts as a control that indicates that sample has been transported throughout the length of test strip (1). Typically dry porous carrier (2) is 55 mm. in length, and 6
mm. wide. All parameters on dry porous carrier (2) may be changed but the width will not affect the sensitivity (provided it is above A4 mm, where edge drag effects come in to play). Absorbent pad (7) is placed upon dry porous carrier (2) opposite conjugate pad (8) such that capillary action can draw sample from first end (3) to second end (4) thereby transporting sample through first reaction site (5), and second reaction site (6). Absorbent pad (7) can be fabricated from any material that has a propensity to wick liquid such as a sponge or paper towel. Located on the opposite end of test strip (1) it can be seen that receiving member (9) is disposed on top of conjugate pad (8). Receiving member (9) is preferably 10 mm long and 5 mm. in width. Receiving member (9) is the first contact point for sample added to test strip (1).

[00100] Referring to FIG. 2, it can be seen that the dry porous carrier (2) of test strip (1) can have a backing (10) equal in length and width to dry porous carrier (2). Backing (10) may be added to add tensile strength to porous carrier (2) and may be made out of any plastic material. It can also be seen that absorbent pad (7) is placed at second end (4) upon dry porous carrier (2) opposite conjugate pad (8). Absorbent pad (7) may be longer than conjugate pad (8), in the range of 4 to 15 mm, in an exemplary embodiment 10 mm in length. Preferably absorbent pad (7) is about 5 mm wide. Absorbent pad (7) may be thicker than conjugate pad (8). It can also be seen that receiving member (9) is located closer to first end (3) and may over hang the end of conjugate pad (8). Receiving member (9) may be between 5 mm to 15 mm in length, in an exemplary embodiment 10 mm. Receiving member (9) is preferably 5 mm wide.

[00101] Referring to FIG. 3, it can be seen that one embodiment of the device comprises a housing, casing, or substrate (11) of elongate rectangular form is of hollow construction. Housing (11) may be between 30 mm to 50 mm wide, in an exemplary embodiment about 37 mm wide. Housing (11) may be between 55 mm. to about 70 mm. in length, preferably about 65 mm. It can be seen that housing (11) also has at its center a divider (12). Substrate (11) can be made out of any material capable of retaining a liquid such as plastic or glass, preferably, substrate (11) is made out of plastic. Divider (12) is positioned on top of substrate (11) in order to form first well (13) and second well (14). Divider (12) is preferably positioned in the center of substrate (11) and runs from the top end (15) to the bottom end (16).
of substrate (11). Divider (12) is preferably positioned so that well (13) is the same size as well (14). In an exemplary embodiment, well (13) and well (14) are large enough so that each well can hold one test strip of the present invention. In the preferred embodiment of the present invention, substrate (11) comprises one divider (12), which makes two wells of equal size, where each well will hold one test strip (1). First well (13) is designed to hold first test strip (20). Second well (14) is designed to hold second test strip (21).

[00102] FIG. 3, also shows that at least one aperture (17) may be positioned around the edges of substrate (11). A cap (18) may be fitted onto substrate (11) and can abut against and fit into apertures (17). Furthermore, FIG. 3 also shows that at least one catch (19) positioned within each well for holding test strip (21) in place. Although numerous catches can be placed inside the wells, preferably, each test strip is given four catches (19) so that test strip (21) is held substantially in place. In this respect, the internal constructional details of housing (11) provides pressure to the test strip components which allows for adequate flowability of sample and analyte throughout the overlapping test strip components.

[00103] FIG. 3, also shows first test strip (20) contains a first receiving member (22) disposed upon a first conjugate pad (23), which is in turn is disposed upon a first dry porous carrier (24). Although not shown in FIG. 3, first dry porous carrier (24) comprising a matrix through which sample can pass by capillarity carrying a first mobile specific binding reagent and a first analyte, as well as complexes thereof. Dry porous carrier (24) comprises two reaction sites, a first reaction zone (25) located towards the middle of first test strip (20). First reaction site (25) comprises a first immobilized specific binding reagent capable of immobilizing a first chromogenic mobile specific binding partner when first analyte is absent in the sample. Dry porous carrier (24) further comprises a second reaction site (26) located downstream between the first reaction zone (25) and the first absorbent pad (27). Second reaction site (26) comprises an immobilized binding reagent capable of immobilizing immunochemicals. Located towards the top end (15) of substrate (11), and downstream on the first dry porous carrier (24) is a first absorbent pad (27). The first test strip (20) is designed similar to the test strip of FIGS. 1 & 2, therefore, sample applied to test strip (20) will first travel through the device in the same fashion.
A negative sample will form a visible line on in first reaction zone (25). In a competition type reaction of the preferred invention, a visible line will form in the second reaction zone (26) as an indication that the sample has traveled through test strip (20).

[00104] FIG. 3, also shows second test strip (21) is similar in size and shape to first test strip (20). It can be seen that second test strip (21) contains a second receiving member (28) disposed upon a second conjugate pad (29), which is in turn disposed upon a second dry porous carrier (30). Second dry porous carrier (30) comprises a matrix through which sample can pass by capillarity carrying a second mobile specific binding partner and a second analyte. A third reaction zone (31) is located towards the middle of second test strip (21). Third reaction site (31) comprises a second immobilizing specific binding reagent capable of immobilizing a second mobile specific binding reagent in relation to the absence of analyte in a sample moving by capillary action, transporting the second chromogenic mobile specific binding reagent through the chromatographic test strip.

[00105] Located towards the top end (15) of substrate (11) is a second absorbent pad (33). A fourth reaction zone (32) is located downstream of the third reaction zone (31) between second absorbent pad (33) and third reaction zone (31). Fourth reaction site (32) comprises a binding reagent capable of immobilizing immunochemicals. Second test strip (21) is designed similar to the test strip of FIGS. 1 & 2 therefore, sample applied to test strip (21) will first travel through the device in the same fashion.

[00106] Although they are similar in size and construction, there are some important differences in the two strips, which are not shown in FIG. 3. First, conjugate pad (23) in test strip (20) is preferably manufactured to contain a first chromogenic mobile specific binding reagent capable of binding to a first analyte. In an exemplary embodiment, the chromogenic mobile specific binding partner in conjugate pad (23) in test strip (20) is capable of specifically binding to lambda monoclonal light chains. Conversely, second conjugate pad (29) can be easily manufactured to contain a second chromogenic mobile specific binding reagent capable of binding to a second analyte. In an exemplary embodiment, the chromogenic mobile
specific binding reagent in second conjugate pad (29) is capable of specifically binding to kappa monoclonal light chain.

[00107] Referring to FIG. 3, the bottom end (16) and top end (15) of well (13) and well (14) respectively may be recessed to accommodate first end (3) and second end (4) within housing (11). Liquid sample applied to receiving members (22) can pass freely into conjugate pad (23) rapidly saturating conjugate pad (23). In turn, conjugate pads (23) is in liquid permeable contact with strip of first dry porous carrier (24). Upon saturation of first conjugate pad (23), liquid sample begins to permeate through the porous dry carrier (24) making it wet. Housing (11) is designed with divider (12) to ensure that first test strip (20) is not contaminated with liquid sample from second test strip (21) within housing (11). Divider (12) solves the problem of cross-contamination between test strips (20) and (21) in the same housing (11). Cross-contamination occurs when liquid sample saturates either test strip (20) or (21) and leaches from one test strip to the other with in housing (11). Leaching liquid may escape from the first test strip and carry along with it immunochemicals, and labeled specific binding reagent specific to that test strip, to the second test strip. Such cross contamination may provide false identification between classes of free light chains. Housing (11) is designed to ensure that liquid sample applied to receiving member (28) does not mix with sample applied to receiving member (22). FIG. 3, does not show backing (10) which would be positioned underneath first dry porous member (24) and second dry porous material (30). Since substrate (11) may provide adequate support for first dry porous member (24), and second dry porous material (30), backing (10) may not be included in all embodiments described herein.

[00108] Referring to FIG. 4A, cap (18) is fitted onto housing (11)(not shown). When cap (18) is fitted onto portion (11) of the housing, it covers first test strip (20), as well as second test strip (21), and becomes the upper face (37) of housing (11). Incorporated into upper face (37) is first window (33) and first gap (34). The first reagent containing detection zone (25), and second reaction zone (26) in first test strip (20), are positioned to be in window (33). When the device is used, liquid sample is added through gap (34). The sample immediately wets the first receiving member (22) of the first test strip (20). Although not visible in FIG. 4A, a sample
will travel through first receiving member (22), through first conjugate pad (23), through dry porous member (24) and travel by capillarity through the test strip (20) through the area visible in first window (33). A negative sample for lambda will show a visible banding pattern at the first reaction site (25). A visible banding pattern will form at second reaction zone (26) as an indication that sample has correctly passed through first test strip (20).

[00109] Incorporated into upper face (37) is second window (35) and second gap (36). The third reagent containing detection zone (31), and fourth reaction zone (32) in second test strip (21), are positioned to be in second window (35). When the device is used, liquid sample is added through gap (36). The sample immediately wets second receiving member (28) of the second test strip (21). Although not visible in FIG. 4A, as sample will travel through second receiving member (28), through second conjugate pad (29), through second dry porous member (30) and travel by capillarity through the test strips through the area visible in second window (35). A negative sample for kappa will show a visible banding pattern at third reaction site (31). A visible banding pattern will form at fourth reaction zone (32) as an indication that sample has correctly passed through second test strip (21).

[00110] Referring to FIG. 4B, an alternate embodiment is illustrated wherein a single gap (34) reveals a receiving member (22) common to both strips (20) and (21). This embodiment creates efficiency of application (only one sample need be applied).

[00111] Referring to FIG. 5, an exemplary test device comprises a flat rectangular cassette (38), incorporating a first gap (34), a first window (33), a second gap (36) and a second window (35). The exploded view clearly shows at least one test strip of the present invention being positioned within cassette (38).

[00112] In operation, an aqueous sample can be applied through gap (34), e.g. by means of a syringe or pipette, to saturate first porous receiving member (22) which passes sample to first conjugate pad (23) containing labeled reagent which can be taken up by the sample. Thereafter, the aqueous sample can permeate the first test strip and, after an appropriate time, the test result can be observed through first window (33). Similarly, an aqueous sample can
be applied through second gap (36), e.g. by means of a syringe, to saturate second porous receiving member (28) which passes sample to second conjugate pad (29) which contains labeled reagent which can be taken up by sample. Thereafter, the aqueous sample can permeate second test strip (21) and, after an appropriate time, the test result can be observed through second window (35). FIG. 5 further shows pin (39), which is designed to snap into aperture (17) to hold the top face (37) to the bottom plate (40). FIG. 5 also shows inner well (41), which optimizes pressure in component system, and ensures optimal flowability of sample throughout test strip components.

[00113] The following examples are given for the purpose of illustrating the present invention and are not intended to limit the scope in any way.

EXAMPLE 1

[00114] Inoculated Goat Serum is Affinity purified against human BJP extract, suspended in a buffered saline solution and conjugated to 40 nm Gold colloid particles (Veda Labs, Alencon-France) by the following protocol. A A40 nm colloidal gold sol solution (externally Quality controlled by EM) is adjusted to pH 6 using 0.2M Sodium Hydroxide solution (using a Beckmann pH meter). The antibodies are introduced in the sol at circa 5-micrograms/ml concentration and incubated for 10 minutes. 10% Bovine serum albumin (Bayer fraction V) in tris buffered saline is then added to a final concentration of 0.2%. The solution is centrifuged for 30 minutes at 4° C. The supernatant is discarded and the pellet resuspended in BSA in Tris buffered saline. The suspension is then introduced into a glycerol gradient column and centrifuged for 45 minutes at 4° C. The purified band is then extracted from the gradient column.

EXAMPLE 2

[00115] Basic Manufacturing Procedure: Inoculated Goat Serum is Affinity purified against human BJP extract, suspended in a buffered saline solution and conjugated to 40 nm Gold colloid particles. Conjugate pad material is liberally soaked in Conjugate solution and cut to about 7 mm length then affixed to CN membrane. Absorbent pads are attached to top and
base of the device to allow sample introduction and osmotic conductance. Purified Human Bence-Jones Protein is suspended in Buffered saline and applied in a 1 mm line on the CN strip (test zone). This Antiserum is a Goat Polyclonal Anti-Human Free and Bound Light chain (But monoclonal or different host animal would be equally adequate). A 1 mm strip of Buffered saline suspended Protein A is applied at the control line. Strips are cut to a 6 mm width and placed in the cassette.

**Sandwich Assay Devices**

[00116] Referring to the drawings, FIGS. 6a, 6b, and 7 depict an exemplary joint assay test device (51) for the detection of free light chains (kappa and/or lambda), free and bound antibodies (kappa and/or lambda), and immunochemicals in urine sample (66) (treated and/or untreated) wherein a chromogenic mobile specific binding partner is impregnated and dried into a conjugate pad (52) by soaking the conjugate pad (52) in a solution containing the chromogenic mobile specific binding partner.

[00117] The chromogenic mobile specific binding partner is selected from anti-free and bound kappa and anti-free and bound lambda antibodies conjugated with a visible colloidal metal. Although other colloidal metals that one of skill in the art would use to bind to an antibody to make a visible color may work, gold provides an easily discernable red band in relation to the presence of analyte. The chromogenic mobile specific binding partner may be one conjugated monoclonal antibody or a conjugated monoclonal antibody cocktail.

[00118] The device (51) may comprise a length of substrate material (53) upon which cellulosic membrane (54) is disposed. Cellulosic membrane (54) has a first end (55) at which chromatographic urine transport begins and a second end (56) at which chromatographic solvent transport ends. The length of cellulosic membrane (54) comprises a first reaction site (57), a second reaction site (58), and a control reaction site (59). Typically cellulosic membrane (54) is 60 mm. in length, and 6 mm. wide. All parameters on the cellulosic membrane (54) may be changed but the width will not affect the sensitivity (provided it is above 4 mm, where edge drag effects come in to play). Absorbent pad (60) is placed upon cellulosic membrane (54)
opposite conjugate pad (52) such that capillary action can draw urine (66) from first end (55) to second end (56) thereby transporting urine (66) through reaction site (57), second reaction site (58), and control site (59). Absorbent pad (60) can be fabricated from any material that has a propensity to wick liquid such as a sponge or paper towel material and is typically 17 mm. long and 6 mm. wide. The size and thickness must be such that it provides enough capillarity to transport urine (66) from first end (55) to second end (56).

[00119] The first reaction site (57) is impregnated with a first immobilized specific binding reagent capable of reaction with and the immobilization of the chromogenic mobile specific binding partner when analyte such as free light chain is present in urine (66) sample. In a joint assay (52) the immobilized specific binding reagent is an anti-free light antibody capable of binding free kappa or lambda light chains in untreated urine samples harboring free light chains. The second reaction site (58) is downstream of the first reaction site (57) and is impregnated with a second immobilizing specific binding factor. Typically, the second immobilizing specific binding factor is anti-free and bound antibody capable of detecting whole antibodies in untreated urine samples harboring whole free and bound antibodies. The control reaction site (59) is further downstream than reaction site (58) and is impregnated with a third immobilizing specific binding factor. Typically a chemical capable of specifically binding to immunochemicals, such as Protein A is used for it is capable of effectively collecting and immobilizing antigen-antibody complexes by binding to the Fc section of immunoglobulins. Control reaction site (59) acts as a positive control and is relied upon to indicate that capillary action has carried test urine (66), chromogenic mobile specific binding partners, and analyte/chromogenic mobile specific binding partner complexes thereof throughout the length of the chromatographic test strip.

[00120] Referring now to FIG. 6c, an exemplary kit is shown. Device (52) is obtained within reaction tube (65) having a cap (63). Reaction tube (65) has line (64) to ensure the correct aliquot of urine (66) is added to reaction tube (65). When device (52) is to be used, the device is removed from reaction tube (65). Urine (66) is added to line (64) to ensure the proper amount of urine (66) aliquot. Urine (66) may be untreated urine which means that it is urine collected directly from a test individual. Urine (66) is typically obtained by collecting a
sample midstream from a urinating individual, and then adding an aliquot to reaction tube (65). Although the urine (66) aliquot may fall within the range of 100 microliters to 1 milliliter, 300 microliters is optimal for the instant invention. Upon the addition of urine aliquot (66) device (52) is placed back into reaction tube (65). Typically Cap (63) is reapplied to reaction tube (65) and the test is allowed to run. The cap (63) ensures a seal on reaction tube (65) providing for an improved disposal of biological waste, reducing the contact between the person using the kit and the biological chemicals and waste therein.

[00121] A method of use for device (51) of FIGS. 6a, 6b, and 7 comprises obtaining cellulosic membrane (54) and placing it laterally upon a surface. Conjugate pad (52) is placed upon first end (55), such that conjugate pad (52) is placed adjacent to first reaction site (57). Absorbent pad (60) is next placed on the opposite end of cellulosic membrane (54) upon second end (56) adjacent to control reaction site (59). A second absorbent pad (60) may be upon conjugate pad (52) to facilitate contacting test urine (66) with conjugate pad (52). The device (51) is next contacted with between 200 microliters to 1 ml. and optimally 300 microliters of untreated urine (66) at conjugated pad (52) such that untreated urine (66) will be transported or wicked through the length of cellulosic membrane (54) to absorbent pad (60) located at second end (56) by capillary action. When the untreated urine (66) passes into conjugate pad (52) the chromogenic mobile specific binding partner goes into solution and will react with antigen in an antigen positive urine sample. Antigen means free light chains.

[00122] When antigen is present a complex called the analyte/chromogenic mobile specific binding partner complex is formed. Typically antibody cocktails containing chromogenic monoclonal antibodies will be put in conjugate pad (52) for the detection of multiple analytes, forming multiple complexes. Such complexes are mobile and will migrate through cellulosic membrane (54) towards absorbent pad (60) due to the capillary action and flow of urine (66) throughout device (52).

[00123] The first reaction site (57) comprises an immobilized specific binding reagent impregnated onto the cellulosic membrane (54). Typical immobilized specific binding reagents include antiserum such as polyclonal anti-free and bound antibodies. In analyte positive
samples antiserum binds to the complex thus immobilizing the complex resulting in a high
collection of chromogenic complex reagent in the first reaction site. When a high
concentration of chromogenic complex reagent becomes immobilized in the first reaction site a
visible band is formed.

[00124] The second reaction site is downstream from the first reaction site and is
impregnated with antiserum capable of immobilizing chromogenic mobile specific binding
partner complexed with whole antibody. When whole antibody antigen is present in urine (66),
and a complex is subsequently formed in conjugate pad (52), reaction site (58) containing
polyclonal anti-free and bound whole antibodies will immobilize said antibodies making them
visible in high concentrations.

[00125] The third control reaction site (59) is located downstream of reaction site
(58) and comprises a specific binding reagent capable of immobilizing the chromogenic mobile
specific binding partner upon contact. Control reaction site (59) acts as an indicator that the test
has worked and that at least urine (66) and mobile specific binding partner have been transported
through the chromatographic strip. Control reaction site (59) will also immobilize complex that
did not bind to first reaction site (57) and/or second reaction site (58). A high concentration of
either bonded complex, or non-complexed mobile specific binding partner at control reaction site
(59) will form a visible band indicating that the test has worked correctly.

[00126] Device (52) is preferably 60 mm. in length and 6 mm. wide. First reaction
site (57) containing a first specific immobilizing reagent impregnated into membrane (54) is
approximately 1 mm. in width, and 6 mm. in length as it extends across membrane (54). Second
reaction site (58) and control reaction site (59) are striped adjacent to the first reaction site (57)
approximately and respectively 5 mm. downstream from first reaction site (57). Conjugate pad
(52) is typically made of glass paper and is approximately 5 mm. long and has a width of 6 mm.
extending across membrane (54). Absorbent pad (60) is approximately 20 mm. long and is also 6
mm. wide extending across the width of membrane (54).
A variety of sandwich-type assay devices including dried labeled reagents and preferably including colloidal particle labeled reagents may be produced according to the invention. It is frequently desirable to avoid premature contact of analyte and sample materials with the reagents and contact of the reagents with each other. Thus, the relative mobility of the sample components and the various reagents or the site relationship between the zones may be selected such that the reagents and sample components mix at only the times and locations desired. U.S. Pat. No. 4,960,691 herein incorporated by reference discloses various methods and devices for conducting chromatographic solvent transport assays where it is desired to avoid contact of a labeled first reagent material (such as an anti-human immunoglobulin antibody) with sample material (such as serum) prior to the time at which the analyte antibody is immobilized against solvent transport at a reaction zone. Other non-analyte antibodies contained in the serum sample are cleared from the third zone by chromatographic solvent transport.

**Competitive Assay Devices**

The assay devices described herein for the detection of free light chains, and immunochemicals in an untreated urine sample wherein a chromogenic mobile specific binding partner is impregnated and dried into a pad are also suitable for the practice of competitive binding type assays. According to such methods, the immobilized second reagent is selected, as in sandwich-type assays, so as to specifically bind with the analyte of interest. The labeled mobile specific binding partner, however, is selected to be a specific binding analogue of the analyte that will bind competitively with the immobilized specific binding reagent. In carrying out competition type assays according to the invention, it is generally not necessary that the analyte and the colloidal particle labeled reagent be prevented from contacting each other prior to their contacting the immobilized specific binding reagent. Thus, the device may be designed so as to mix the analyte containing untreated urine sample and the chromogenic mobile specific binding partner.

Referring to the drawing, FIGS. 8a and 8b depict a test device (61) for conducting competitive binding assays for the detection of the analyte of interest such as free light chains in an untreated urine (66) wherein a chromogenic mobile specific binding partner is
impregnated and dried into conjugate pad (52). Typically the chromogenic mobile specific
binding partner in the competitive assay is selected from antibodies to the free and bound light
chain classes that have been conjugated with colloidal gold. Device (61) comprises a length of
chromatographic substrate material (62) with a first end (55) at which chromatographic solvent
transport begins and a second end (56) at which chromatographic solvent transport ends. The
length of material (62) comprises a first reaction site (57) and a control reaction site (59). First
reaction site (57) is impregnated with immobilized specific binding reagent, which is typically a
monoclonal light chain selected from free kappa, free and bound kappa, free lambda, and free
and bound lambda. By selecting and utilizing a different immobilized specific binding reagent it
is possible to create four assays in an array, each having a different first reaction site (57). The
control reaction site (59) is downstream of the first reaction site (57) and is impregnated with a
second reagent, which is capable of a selective binding reaction with both the analyte and the
chromogenic mobile specific binding partner and complexes thereof so as to render the analyte
and chromogenic mobile specific binding partner in immobilized form. Protein A is one such
suitable reagent capable of binding immunochemicals and complexes thereof. The device further
comprises an inert substrate (3) to which the length of chromatographic substrate material (62) is
affixed. Typically this inert substrate is simply a backing material to a nitrocellulose membrane
or cellulosic membrane.

[00130] According to a procedure for use of device (61) of FIGS. 8a, 8b, and 8c,
cap (63) is removed from reaction tube (65) the device (61) is removed, an untreated urine (66)
sample aliquot to be tested in an amount ranging between 0.200 ml. to 1.0 ml, optimally 0.300
ml. is added to reaction tube (65). Device (61) is again deposited within the reaction tube such
that first end (55) and conjugate pad (52) is deposited directly into the untreated urine (66). The
cap (63) is replaced. Upon contact of device (61) at its first end (55) into a reaction tube (65) of
urine aliquot (66), the mobile specific binding partners go into solution and begin to flow with
the urine (66). If the analyte of interest is present in urine (66), a reaction occurs where the
analyte of interest and the chromogenic mobile specific binding partner will form a complex.
The urine aliquot (66) then progresses through the length of the chromatographic substrate
material (62) transporting the chromogenic mobile specific binding partner impregnated at the
conjugate pad (52) and/or any complexes thereof to the first reaction site (57). There the analyte and chromogenic mobile specific binding partner compete to bind with the immobilized specific binding reagent for which they are both specifically reactive. If no analyte is present the chromogenic mobile specific binding partner will bind to the immobilized specific binding reagent and a visible line will form. Non-analyte components as well as unbound analyte and chromogenic mobile specific binding partner are transported away from the first reaction site (57) by means of the urine transport which continues until the chromatographic solvent is exhausted or the urine front reaches the second end (56) of the material. At the conclusion of the chromatographic solvent transport, the first reaction site (57) may be observed to determine the presence of chromogenic mobile specific binding partner immobilized at that location. The presence of chromogenic mobile specific binding partner at that location may then be related to the presence of analyte in the sample. Where the chromogenic mobile specific binding partner is labeled with colloidal particles, its presence at the first reaction site, as well as the presence of analyte/chromogenic mobile specific binding partner complex may be observed directly.

[00131] The presence of chromogenic mobile specific binding partner and analyte complexes thereof at control reaction site (59) is also determined to ensure that the test strip has worked properly.

[00132] A typical procedure for using the test kit as shown in FIG. 8c comprises: removing the reaction device from a tube; adding 300 microliters (0.3 ml) urine (66) to the tube; replacing the reaction device; ensuring that the conjugate pad is at the base and that the absorbent pad is at the top; allowing the urine (66) to migrate through the device; waiting 5-10 minutes; and reading the result—the assay is complete where there is clear banding without background coloration on the strip. Clear banding means discernable band formation as substantially shown and described in FIGS. 9a, 9b, 9c, and 9d.

[00133] Referring now to FIGS. 9a, 9b, 9c, and 9d a schematic diagram is shown illustrating how the results for the competitive assay should be interpreted. FIG. 9a shows the absence of a band in the first reaction site (57) indicating a positive sample. FIG. 9a also shows control reaction site (59), which should always have a line in the completed assay—if no control
line is present at reaction site (59) the test should be repeated. FIG. 9b shows clear line formation in first reaction site (57) and a clear line formation in control reaction site (59) indicating respectively a negative result for the analyte of interest, and that the test has worked. FIG. 9c shows a clear line formation at control reaction site (59) and a faint line formation at reaction site (57) illustrating a low positive sample, and that the test has worked. FIG. 9d shows a faint band formation at control reaction site (57) indicating a sample positive for analyte. FIG. 9d also shows clear band formation at the control reaction site indicating that the test has worked. In samples comprising very low BJP titers a faint (ghost) line may be observed. Under these circumstances the sample should be concentrated prior to immunofixation.

[00134] The test may be used as a general screen of all potential myeloma samples, or as a more general screen for all senior population and renal referral urines. It may also be used after electrophoresis, where the presence of a band in the gamma/beta region is not easily discernible.

EXAMPLE 3

Competitive Immunoassay Dipstick

[00135] Goat antibodies to the Free and Bound light chain classes were conjugated to 40 nm colloidal gold particles and introduced to a conjugate pad of glass paper. A 6 mm wide strip of cellulose nitrate was striped with individual lines of Protein A and urinary derived monoclonal light chain. An absorbent pad was attached to one end of the cellulose nitrate membrane. The glass pad was compressed against the cellulose nitrate membrane and urine seeded with monoclonal urinary derived light chains at concentrations 0 m/L, 10 mg/L, 20 mg/L, 30 mg/L, 50 mg/L, 100 m/L, 500 mg/L and 1000 mg/L. Urines were also seeded with albumin, IgA, IgG and IgM (polyclonal) at concentrations 0 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 50 mg/L, 100 m/L, 500 mg/L and 1000 mg/L. 300 microliters of seeded urine was introduced to the glass pad.

[00136] Results are classified according to the absence of a clear line (positive) on the cellulose acetate where the competitive light chains were striped. Where the result was
unclear an assignation 'G' is recorded. In all cases a line was observed at the Protein A line indicating that the conjugate/conjugate-antigen complex had correctly migrated through the test zone.

<table>
<thead>
<tr>
<th>Class mg/l</th>
<th>Kappa</th>
<th>Lambda</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>Albumin</th>
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<td>-</td>
<td>-</td>
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<td>+</td>
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<td>-</td>
</tr>
</tbody>
</table>

**EXAMPLE 4**

**Summary of Clinical Results Test** Sensitivity 10 mg/L Urinary Free Light Chain

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>88%*</td>
<td>100%</td>
</tr>
</tbody>
</table>

[00137] Assay shows no cross reactivity between light chains, with pH, Sg, Albumin or any other physiological factors. *Samples demonstrated to have low levels of polyclonal (para) protein.

[00138] While exemplary embodiments have been described herein, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed.
What is claimed is:

1. A method of screening for a disease state producing bence jones protein, comprising chromatographic testing for bence jones protein presence in unspun or un-centrifuged urine.

2. A method of monitoring for a disease state producing bence jones protein, comprising chromatographic testing for bence jones protein presence in unspun or un-centrifuged urine.

3. A method of adjunctive testing of a disease state producing bence jones protein, comprising chromatographic testing for bence jones protein presence in unspun or un-centrifuged urine.


5. A rapid chromatographic test for detection of free light chains in urine, wherein the rapid test produces results within 5 minutes, with a sensitivity greater than about 88% (for approximately 10mg/L urinary free light chain) and a specificity of greater than about 99%.

6. A rapid chromatographic test for detection of free light chains in urine, including a reaction device for kappa, and a reaction device for lambda.

7. A method of diagnosing an indicator for a medical condition precipitating free light chains in urine, the method comprising exposing a chromatographic test device to untreated or unspun urine, wherein the chromatographic test device indicates presence of said free light chains in the urine.
8. The method in accordance with claim 7, wherein the medical condition is one or more of Multiple Myeloma, micromolecular myeloma, Waldstrom’s Macroglobulinemia, Chrime Lymphatic Leukemia, Amyloidosis, lupus erythematosus, Monoclonal Gammopathies of Undetermined Significance, Fasone’s Syndrome, Renal Insufficiency/Renal Failure, Renal Failure due to Diabetes, Renal failure due to hypertension, and acute rheumatoid arthritis.

9. The method in accordance with claim 7, wherein the free light chain is one or more of a monoclonal free light chain and a polyclonal free light chain.

10. A chromatographic testing cartridge configured to detect free light chains in urine, comprising at least one lateral strip pathway.

11. The chromatographic testing cartridge in accordance with claim 10, wherein lateral flow begins at one origin (sample application) and proceeds laterally along at least two distinct pathways.

12. A chromatographic home testing cartridge configured to detect free light chains in urine, comprising a conjugate pad or a lateral flow dipstick.
13. A method for determining the presence of an analyte in urine sample, comprising:

providing a pad comprising a chromogenic mobile specific binding partner for an analyte;

providing a chromatographic test strip comprising a matrix through which a urine test sample can flow by capillarity action wherein said chromatographic test strip comprises at least two reaction sites;

a first reaction site comprising a first immobilized specific binding reagent capable of immobilizing said chromogenic mobile specific binding partner in relation to the presence of the analyte in the urine sample; and

a control reaction site comprising a specific binding reagent capable of immobilizing said chromogenic mobile specific binding partner;

contacting said conjugate pad to said chromatographic test strip such that said first reaction site lies between said conjugate pad and said control reaction site;

contacting said chromatographic test strip with an absorbent pad such that said absorbent pad is positioned opposite said conjugate pad and such that both said first reaction site and control reaction site lie in-between said conjugate pad and said absorbent pad;

developing said chromatographic test strip by applying urine sample suspected of containing said analyte thereto thereby allowing the same to contact said chromogenic mobile specific binding partner to form an analyte/chromogenic mobile specific binding partner complex whereby capillarity carries the urine test sample along the strip to the first reaction site containing said immobilized specific binding reagent and said control reaction site comprising said specific binding partner;

determining the presence of analyte in the urine test sample by detecting the presence of chromogenic complex at said first reaction site;
determining if migration has occurred by detecting the presence of chromogenic complex
at said control reaction site; wherein detection may be made by observation of color at the
control reaction site.
14. The method of claim 13 further comprising a second reaction site positioned in-between said first reaction site and said control reaction site capable of immobilizing said chromogenic mobile specific binding partner in relation to the presence of whole antibody in said urine.

15. The method of claim 13 wherein said analyte is selected form the group consisting of free kappa chains, free and bound kappa, free lambda, and free and bound lambda.

16. The method of claim 13 wherein said urine sample is untreated urine.

17. The method of claim 13 wherein said mobile specific binding partner is at least one conjugated monoclonal antibody.

18. The method of claim 13 wherein said chromogenic mobile specific binding partner is selected from the group consisting of conjugated anti-free and bound kappa antibody and conjugated anti-free and bound lambda antibody.

19. The method of claim 13 wherein said immobilized specific binding reagent is selected from the group consisting of free kappa, free and bound kappa, free lambda, free and bound lambda for performing a competitive analysis.

20. The method of claim 13 wherein said immobilized specific binding reagent is selected from the group consisting of anti-free kappa antibody and anti-free lambda antibody.

21. The method of claim 13 wherein said specific binding reagent is Protein A for the detection of immunochemicals.

22. The method of claim 13 wherein said second reaction site comprises a second specific binding reagent selected from the group consisting of anti-free and bound kappa antibody and anti-free and bound lambda antibody for the determination of the presence of whole antibody.
23. The method of claim 13 wherein said assay is a sandwich assay and the step of determining the presence of analyte in urine further comprises visualization of a band at said first and second reaction site.

24. The method of claim 13 wherein the step of determining the presence of analyte in urine further comprises visualization of said first and said control reaction site, wherein the absence of band formation at said first reaction site indicates a positive result and the visualization of a band at said first reaction site indicates a negative result and wherein the visualization of band formation at said control reaction site indicates that the test has worked in competitive assay.
25. A device for the detection of analyte in urine comprising:

   a first pad comprising a chromogenic mobile specific binding partner capable of binding to an analyte;

   a chromatographic test strip comprising a matrix through which urine can pass by capillarity carrying said mobile specific binding partner and said analyte, wherein said chromatographic test strip comprises three reaction sites,

   a first reaction site comprising an immobilized specific binding reagent capable of immobilizing said chromogenic mobile specific binding partner in relation to the presence of the analyte in the urine sample,

   a second reaction site comprising a second immobilizing specific binding reagent capable of immobilizing said chromogenic mobile specific binding partner in relation to the presence of the analyte in the urine sample,

   a third control reaction site comprising a third immobilizing specific binding partner capable of immobilizing said mobile specific binding partner in relation to the capillary action transporting said chromogenic mobile specific binding partner through said chromatographic test strip;

   a second, absorbent pad disposed upon said chromatographic test strip such that said absorbent pad is positioned opposite said first pad and such that said first reaction site, second reaction site, and said third reaction site lie in-between said first pad and said absorbent pad.

26. The device of claim 25 wherein said urine is untreated human urine.

27. The device of claim 25 wherein said analyte is selected from the group consisting of free kappa chains, free and bound kappa, free lambda, and free and bound lambda.

28. The device of claim 25 wherein said chromatographic test strip is a porous material.
29. The device of claim 25 wherein said chromogenic mobile specific binding partner is at least one conjugated monoclonal antibody.

30. The device of claim 25 wherein said chromogenic mobile specific binding partner is a conjugated monoclonal antibody cocktail.

31. The device of claim 25 wherein said chromogenic mobile specific antibody is selected from the group consisting of conjugated anti-free and bound kappa antibody and conjugated anti-free and bound lambda antibody.

32. The device of claim 25 wherein said immobilized specific binding reagent is anti-free kappa antibody.

33. The device of claim 25 wherein said third immobilizing specific binding partner is Protein A.