US007279136B2

(12) **United States Patent**
Takeuchi et al.

(10) **Patent No.: US 7,279,136 B2**
(45) **Date of Patent: Oct. 9, 2007**

(54) **METERING TECHNIQUE FOR LATERAL FLOW ASSAY DEVICES**

(76) Inventors: **James M. Takeuchi**, 4714 Calibre Creek Pkwy., Roswell, GA (US) 30076; **Xuedong Song**, 1135 Crabapple Lake Cir., Roswell, GA (US) 30076; **Kaiyuan Yang**, 6155 Ivey Hill Dr., Cumming, GA (US) 30040; **Ning Wei**, 530 Summerhill Dr., Roswell, GA (US) 30075; **Shawn R. Feaster**, 525 Stedford La., Duluth, GA (US) 30097

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **11/301,631**

(22) Filed: **Dec. 13, 2005**

(65) **Prior Publication Data**

US 2007/0134811 A1 Jun. 14, 2007

(51) **Int. Cl.**

G01N 21/00	(2006.01)
G01N 31/22	(2006.01)
G01N 15/06	(2006.01)
G01N 33/53	(2006.01)
G01N 33/566	(2006.01)
G01N 31/00	(2006.01)
B01L 3/00	(2006.01)
B32B 5/02	(2006.01)
C12Q 1/00	(2006.01)
C12M 1/36	(2006.01)
C12M 1/34	(2006.01)
C12M 3/00	(2006.01)

(52) **U.S. Cl.** **422/100**; 422/55; 422/56; 422/57; 422/61; 422/68.1; 422/101; 435/4; 435/7.1; 435/286.4; 435/286.5; 435/287.1; 435/287.2; 435/287.7; 435/287.9; 435/288.5; 435/288.7; 436/501; 436/518; 436/17; 436/18; 436/825

(58) **Field of Classification Search** 422/55–57, 422/61, 68.1, 100, 101; 435/4, 7.1, 286.4, 435/286.5, 287.1, 287.2, 287.7, 287.9, 288.5, 435/288.7; 436/501, 518, 17, 18, 825
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,700,623 A 10/1972 Keim

(Continued)

FOREIGN PATENT DOCUMENTS

EP 0566046 A1 10/1993

(Continued)

OTHER PUBLICATIONS

PCT Search Report—Jan. 16, 2007.

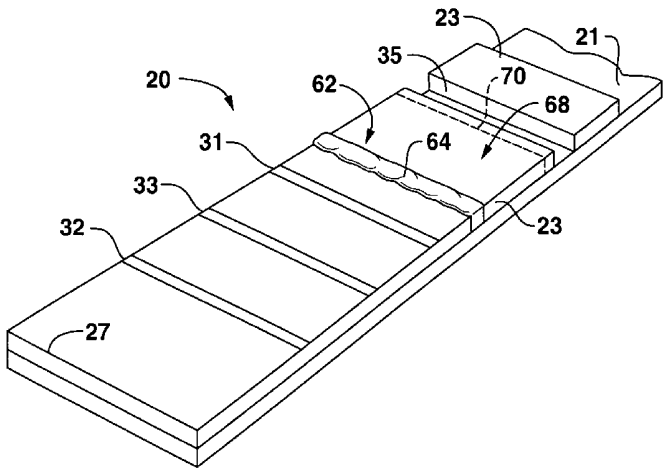
(Continued)

Primary Examiner—Long V. Le
Assistant Examiner—Jacqueline A. DiRamio

(57) **ABSTRACT**

A diagnostic method and associated test kit for detecting an analyte residing in a test sample is provided. A sample membrane is utilized having a collection region and a detection region, the collection region having a known saturation volume for the intended test sample. A barrier is defined between the collection region and the detection region. The collection region is saturated with the test sample having a volume of less than about 100 microliters so that a known volume of the test sample is contained in the collection region. The barrier is removed from between the collection region and detection region of the membrane and a diluent is supplied to the collection region of the membrane to facilitate flow of the test sample from the collection region to the detection region of the membrane.

26 Claims, 4 Drawing Sheets



U.S. PATENT DOCUMENTS

3,772,076 A 11/1973 Keim
4,168,146 A 9/1979 Grubb et al.
4,235,601 A 11/1980 Deutsch et al.
4,366,241 A 12/1982 Tom et al.
4,426,451 A 1/1984 Columbus
4,442,204 A 4/1984 Greenquist et al.
4,537,657 A 8/1985 Keim
4,614,723 A 9/1986 Schmidt et al.
4,742,011 A 5/1988 Blake et al.
4,743,560 A 5/1988 Campbell et al.
4,810,470 A 3/1989 Burkhardt et al.
4,835,099 A 5/1989 Mize et al.
4,857,453 A 8/1989 Ullman et al.
4,889,816 A 12/1989 Davis et al.
4,904,583 A 2/1990 Mapes et al.
4,920,046 A 4/1990 McFarland et al.
4,954,435 A 9/1990 Krauth
4,956,302 A 9/1990 Gordon et al.
4,959,324 A 9/1990 Ramel et al.
4,963,498 A 10/1990 Hillman et al.
4,978,625 A 12/1990 Wagner et al.
4,980,298 A 12/1990 Blake et al.
4,987,085 A 1/1991 Allen et al.
4,999,287 A 3/1991 Allen et al.
5,073,340 A 12/1991 Covington et al.
5,075,077 A 12/1991 Durley, III et al.
5,075,078 A 12/1991 Osikowicz et al.
5,120,643 A 6/1992 Ching et al.
5,145,784 A 9/1992 Cox et al.
5,149,622 A 9/1992 Brown et al.
5,185,127 A 2/1993 Vonk
5,204,063 A 4/1993 Allen
5,208,143 A 5/1993 Henderson et al.
5,208,535 A 5/1993 Nakayama et al.
5,234,813 A 8/1993 McGeehan et al.
5,252,459 A 10/1993 Tarcha et al.
5,264,180 A 11/1993 Allen et al.
5,275,785 A 1/1994 May et al.
5,395,754 A 3/1995 Lambotte et al.
5,416,000 A 5/1995 Allen et al.
5,423,989 A 6/1995 Allen et al.
5,428,690 A 6/1995 Bacus et al.
5,464,741 A 11/1995 Hendrix
5,518,883 A 5/1996 Soini
5,573,909 A 11/1996 Singer et al.
5,573,919 A 11/1996 Kearns et al.
5,585,279 A 12/1996 Davidson
5,589,399 A 12/1996 Allen et al.
5,591,581 A 1/1997 Massey et al.
5,591,645 A 1/1997 Rosenstein
5,602,040 A 2/1997 May et al.
5,607,863 A * 3/1997 Chandler 436/518
5,610,077 A 3/1997 Davis et al.
5,622,871 A 4/1997 May et al.
5,624,537 A 4/1997 Turner et al.
5,637,509 A 6/1997 Hemmilä et al.
5,656,503 A 8/1997 May et al.
5,670,381 A 9/1997 Jou et al.
5,698,406 A 12/1997 Cathey et al.
5,714,389 A 2/1998 Charlton et al.
5,731,147 A 3/1998 Bard et al.
5,736,188 A 4/1998 Alcock et al.
5,753,497 A 5/1998 Bernstein et al.
5,788,863 A 8/1998 Milunic
5,798,272 A 8/1998 Allen et al.
5,798,273 A 8/1998 Shuler et al.
5,922,537 A 7/1999 Ewart et al.
5,945,281 A 8/1999 Prabhu
5,981,294 A 11/1999 Blatt et al.
5,989,924 A 11/1999 Root et al.
5,989,926 A 11/1999 Badley et al.
5,998,221 A 12/1999 Malick et al.
6,004,530 A 12/1999 Sagner et al.
6,030,840 A 2/2000 Mullinax et al.
6,057,165 A 5/2000 Mansour
6,077,669 A 6/2000 Little et al.
6,130,100 A 10/2000 Jobling et al.
6,133,048 A 10/2000 Penfold et al.
6,156,271 A 12/2000 May
6,187,269 B1 2/2001 Lancesseru et al.
6,194,220 B1 2/2001 Malick et al.
6,242,268 B1 6/2001 Wieder et al.
6,258,045 B1 7/2001 Ray et al.
6,261,779 B1 7/2001 Barbera-Guillem et al.
6,270,637 B1 8/2001 Crismore et al.
6,274,324 B1 8/2001 Davis et al.
6,274,384 B1 8/2001 Starzl et al.
6,294,391 B1 9/2001 Badley et al.
6,316,205 B1 * 11/2001 Guan et al. 435/7.1
6,352,862 B1 3/2002 Davis et al.
6,362,011 B1 3/2002 Massey et al.
6,365,417 B1 4/2002 Fleming et al.
6,399,398 B1 6/2002 Cunningham et al.
6,416,642 B1 7/2002 Alajoki et al.
6,436,651 B1 8/2002 Everhart et al.
6,436,722 B1 8/2002 Clark et al.
6,444,423 B1 9/2002 Meade et al.
6,468,741 B1 10/2002 Massey et al.
6,475,805 B1 11/2002 Charm et al.
6,481,453 B1 11/2002 O'Connor et al.
6,524,864 B2 2/2003 Fernandez de Castro
6,582,930 B1 6/2003 Ponomarev et al.
6,585,939 B1 7/2003 Dapprich
6,613,583 B1 9/2003 Richter et al.
6,620,112 B2 9/2003 Klitmose
6,627,459 B1 9/2003 Tung et al.
6,653,149 B1 11/2003 Tung et al.
6,669,908 B2 12/2003 Weyker et al.
RE38,430 E 2/2004 Rosenstein
6,951,631 B1 10/2005 Catt et al.
6,998,273 B1 * 2/2006 Fleming et al. 436/514
7,044,919 B1 5/2006 Catt et al.
7,052,831 B2 5/2006 Fletcher et al.
7,083,939 B2 8/2006 Shull et al.
2002/0042149 A1 4/2002 Butlin et al.
2002/0045273 A1 4/2002 Butlin et al.
2002/0081575 A1 6/2002 Small et al.
2003/0032196 A1 2/2003 Zhou
2003/0113528 A1 6/2003 Moya
2003/0118480 A1 6/2003 Kaylor et al.
2003/0119202 A1 6/2003 Kaylor et al.
2003/0119204 A1 6/2003 Wei et al.
2003/0124739 A1 7/2003 Song et al.
2003/0139886 A1 7/2003 Bodzin et al.
2003/0149348 A1 8/2003 Raskas
2004/0043502 A1 3/2004 Song et al.
2004/0043507 A1 3/2004 Song et al.
2004/0043511 A1 3/2004 Song et al.
2004/0082077 A1 4/2004 Hu
2004/0087034 A1 * 5/2004 Lien 436/514
2004/0106190 A1 6/2004 Yang et al.
2004/0121334 A1 6/2004 Wei et al.
2004/0126833 A1 7/2004 Shull et al.
2004/0151632 A1 8/2004 Badley et al.
2004/0161859 A1 8/2004 Guo et al.
2005/0011759 A1 1/2005 Moerman et al.
2005/0029924 A1 2/2005 Okay et al.
2005/0036148 A1 2/2005 Phelan et al.
2005/0037510 A1 2/2005 Sharrock et al.
2005/0084982 A1 4/2005 Brauner
2005/0109951 A1 5/2005 Fish et al.
2005/0112635 A1 5/2005 Gentle et al.
2005/0112780 A1 5/2005 Song
2005/0124077 A1 * 6/2005 Cole et al. 436/518

2005/0136500	A1	6/2005	Yang et al.	
2005/0175992	A1*	8/2005	Aberl et al.	435/5
2006/0051237	A1*	3/2006	Wang et al.	422/56
2006/0127924	A1	6/2006	Hellyer et al.	
2006/0160078	A1*	7/2006	Cardy et al.	435/6
2006/0246597	A1	11/2006	Feaster et al.	
2006/0246600	A1*	11/2006	Yang et al.	436/514

FOREIGN PATENT DOCUMENTS

EP	0883159	A2	4/1998
GB	2090659	A	7/1982
WO	WO 0050891	A1	8/2000
WO	WO 0063697	A1	10/2000
WO	WO 0078917	A1	12/2000
WO	WO 0117797	A1	3/2001
WO	WO 0198785	A2	12/2001
WO	WO 0198785	A3	12/2001
WO	WO 03008971	A2	1/2003
WO	WO 03008971	A3	1/2003
WO	WO 03025574	A1	3/2003
WO	WO 03058246	A1	7/2003

OTHER PUBLICATIONS

Article—*Flow-Based Microimmunoassay*, Hayes et al., Anal. Chem., vol. 93, No. 24, Dec. 15, 2001, pp. 5896-5902.

Report by David L. Carlberg entitled *High-volume Manufacturing of Lateral Flow Assays*, 3 pages.

Data Sheet for MF-Millipore™ Filters from Millipore, 5 pages.

Product Catalogue—MF-Millipore™ Membrane Filters, 6 pages, www.millipore.com.

Product Information Sheet—Nitrocellulose in Solution from Wolff Cellulosics, 4 pages, www.wolff-cellulosics.de.

Article: *Solvent-Assisted Microcontact Molding: A Convenient Method for Fabricating Three-Dimensional Structures on Surfaces of Polymers*, Enoch Kim et al., Advanced Materials, vol. 9, No. 8, Jun. 1997, pp. 651-654.

Article: *Inkjet Printed Via-Hole Interconnections and Resistors for All-Polymer Transistor Circuits*, Takeo Kawase et al., Advanced Materials, vol. 13, No. 21, Nov. 2, 2001, pp. 1601-1605.

* cited by examiner

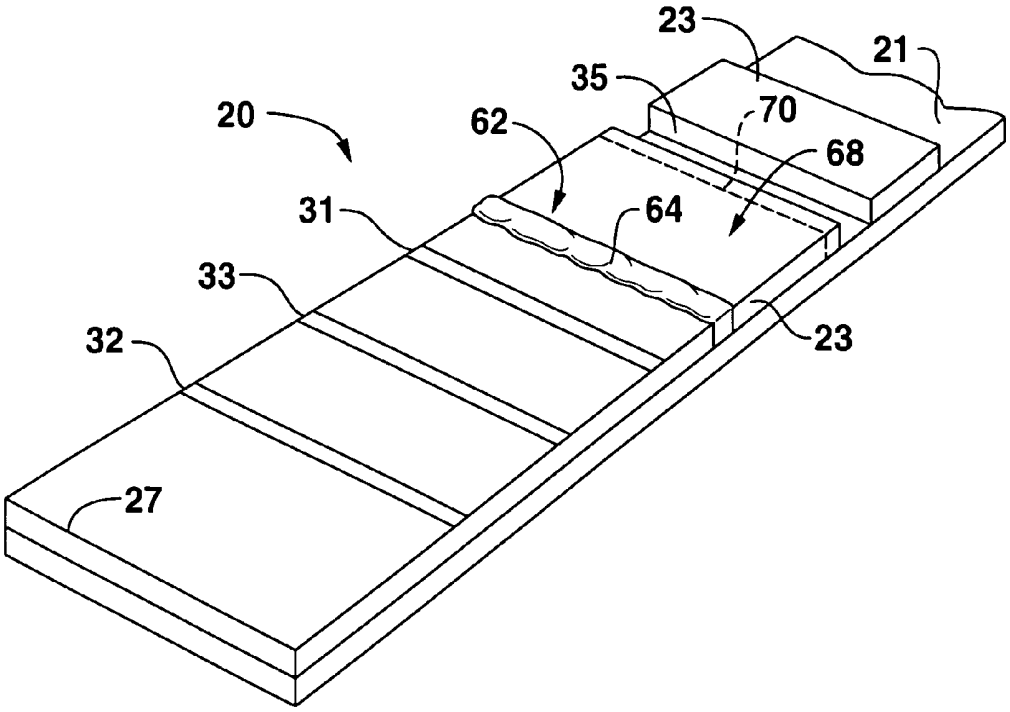

23
21
35
70
20
62
68
31
64
33
23
32
27

FIG. 1

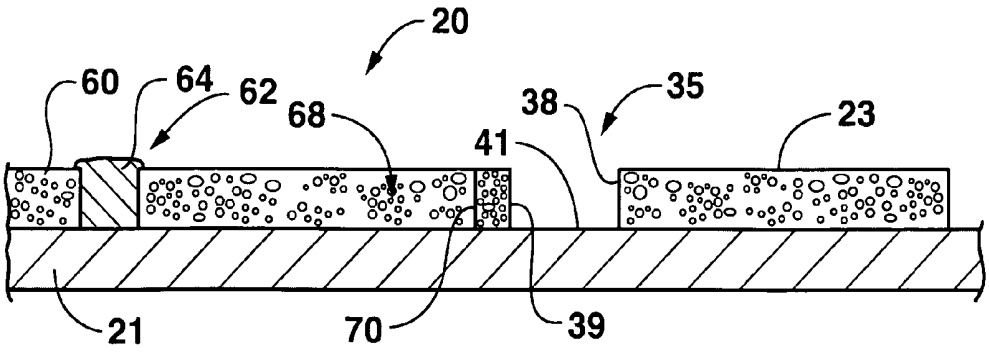

20
60
64
62
68
38
35
41
23
21
70
39

FIG. 2

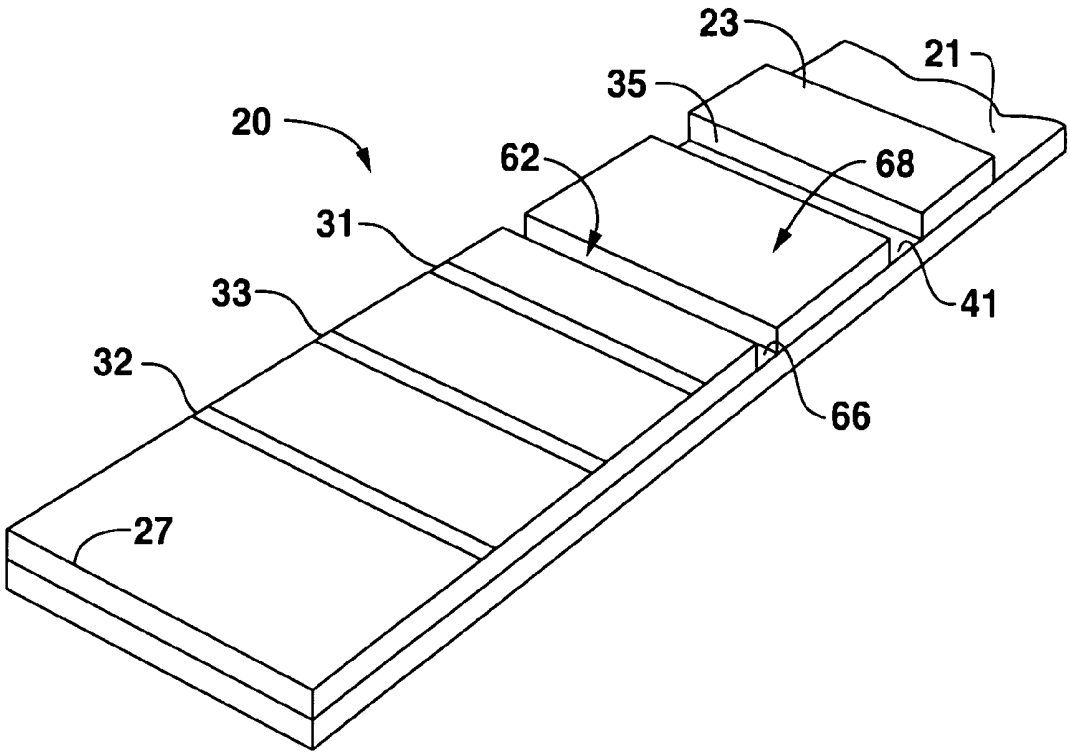


FIG. 3

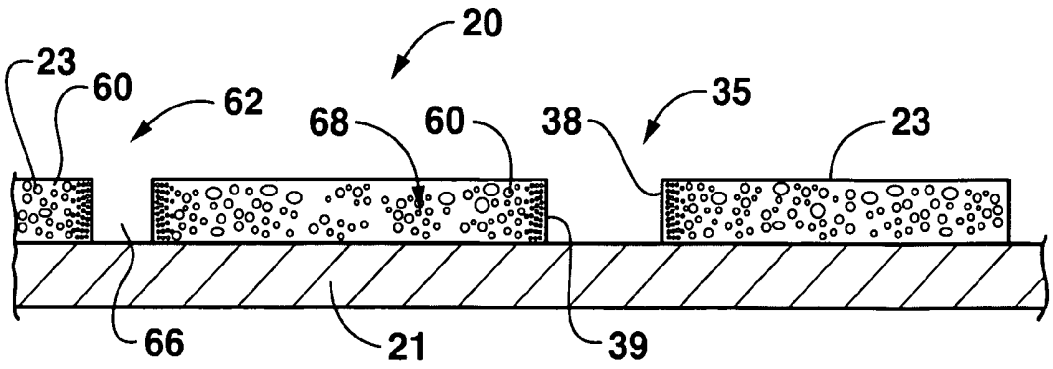


FIG. 4

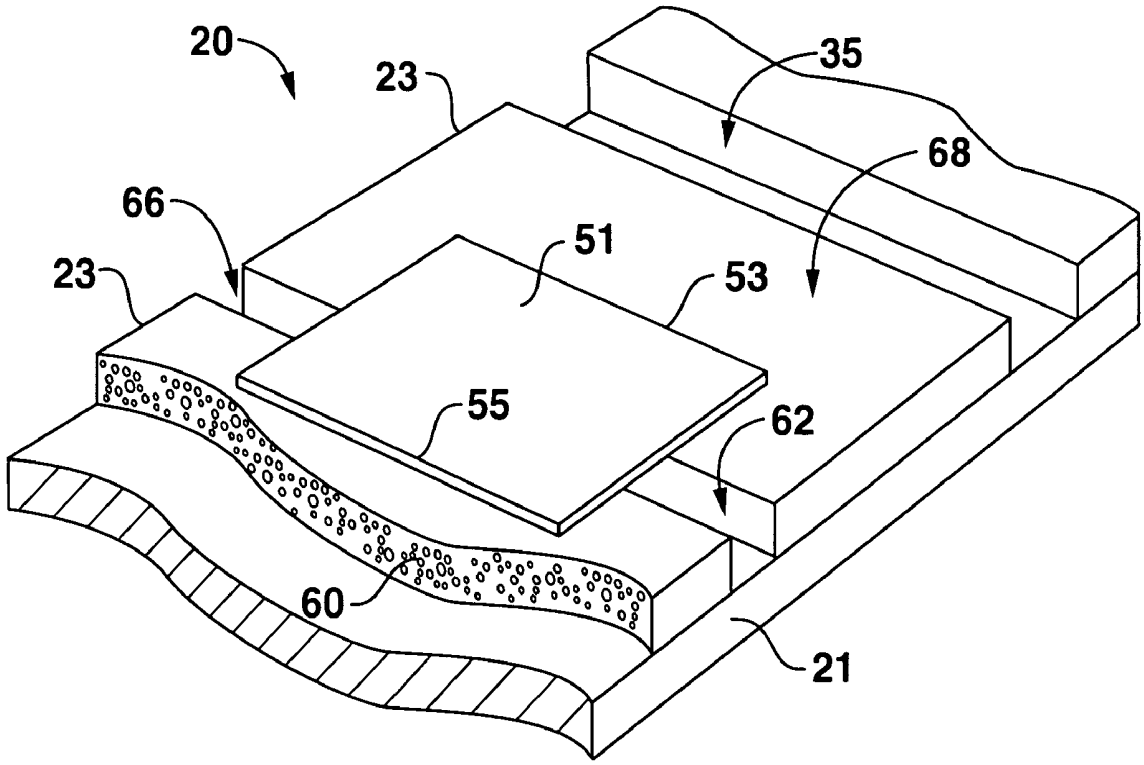


FIG. 5

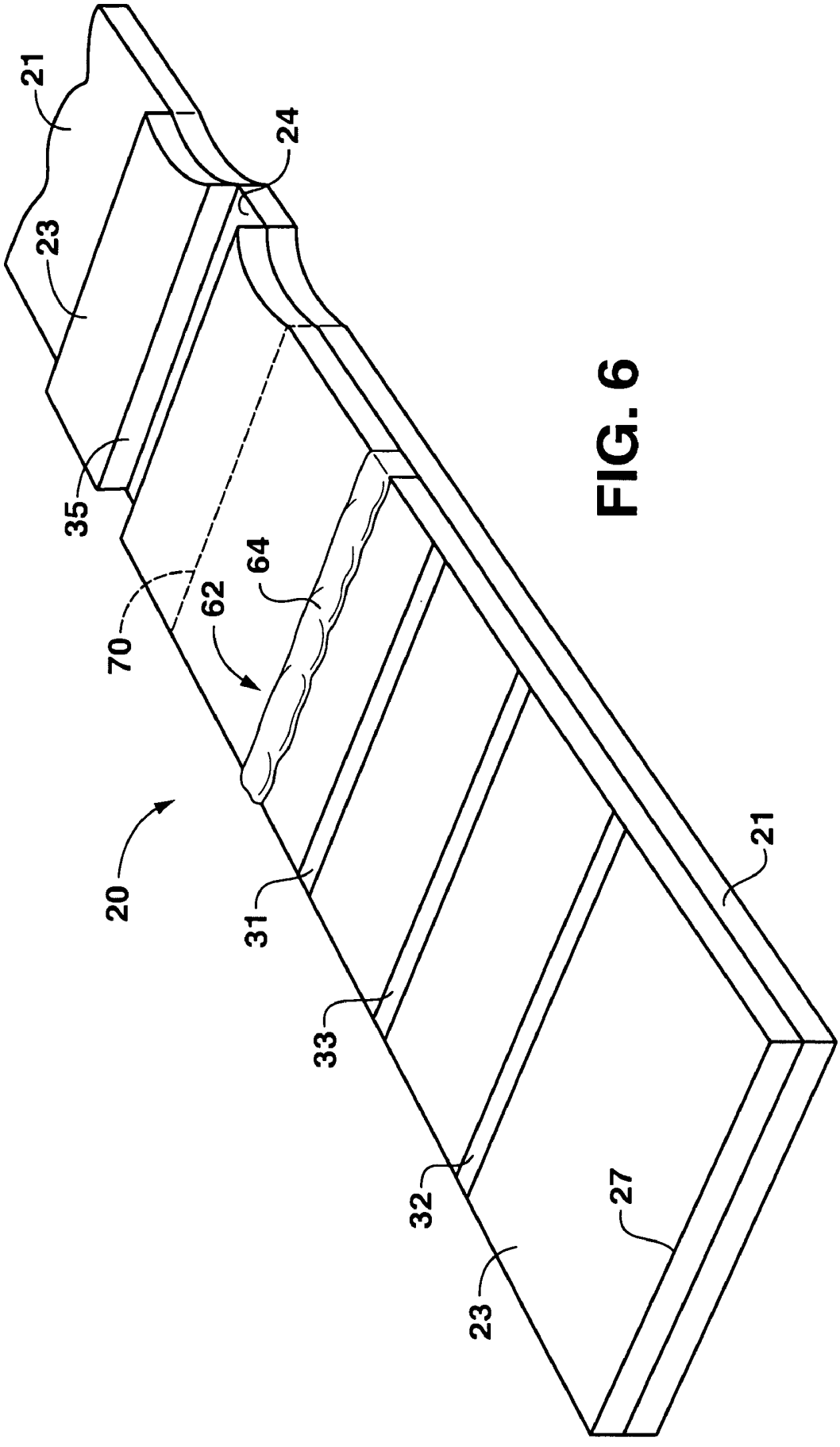


FIG. 6

METERING TECHNIQUE FOR LATERAL FLOW ASSAY DEVICES

BACKGROUND OF THE INVENTION

Test strips are often used for qualitative and quantitative analysis of blood components. The test strips are sometimes constructed so that the sample application area and the detection area are stacked above one another in a vertical axis. However, this type of construction is associated with a number of problems. For example, when the test strip is inserted into an instrument for measurement, the potentially infectious sample material may contact parts of the optical reader and result in contamination. Thus, spatial separation between the sample application area and detection zone is often desired, i.e., lateral flow strips. Most conventional lateral flow strips are designed for test samples that are readily available in large quantities (e.g., urine). However, when the test sample is blood, the collection of a large sample may cause undue pain to the patient. Thus, one technique that has been utilized to accommodate smaller test sample volumes is to "spot" the sample directly only the membrane surface. Thereafter, a diluent is used to wash away the test sample and carry it to the detection zone. Unfortunately, variations associated with sample transfer and diffusion of the sample to the membrane result in a flow that is largely uncontrolled and uneven before reaching the detection zone. This may have an adverse affect on the accuracy of the device because the amount of analyte and/or label captured across the detection zone is not consistent at the time of measurement.

As such, a need currently exists for a simple and efficient technique for metering a low volume test sample to a detection zone of a lateral flow assay device.

SUMMARY OF THE INVENTION

Objects and advantages of the invention will be set forth in part in the following description, or may be obvious from the description, or may be learned through practice of the invention.

In accordance with one embodiment of the present invention, a diagnostic method, and test kit for practice of the method, are provided for detecting the presence of an analyte within a test sample. A test membrane is provided having a collection region and a detection region, the collection region having a known saturation volume for the sample to be tested. A temporary or removable barrier is defined between the collection region and the detection region. The collection region is saturated with a test sample having a volume of less than about 100 microliters so that a known volume of the test sample is contained in the collection region. The barrier is subsequently removed from between the collection region and detection region of the membrane, and a diluent is supplied to the collection region to facilitate flow of the test sample from the collection region to the detection region.

In one embodiment, the barrier may be defined by a soluble composition in a defined band across the width of the membrane, with the soluble composition present throughout the membrane in an amount to prevent migration of the test sample from the collection region to the detection region. The soluble composition may be dissolved by the diluent and is selected so as to not to affect detection of the analyte in the test sample.

In an alternate embodiment, the barrier may be defined by a physical divide or separation in the membrane, such as a channel across the width of the membrane, wherein the step of removing the barrier includes forming a bridge across the divide so that the test sample flows from the collection region to the detection region with the diluent.

The invention is not limited to any particular test sample, and in one embodiment, the test sample is whole blood. Plasma, serum, or both may be separated from the whole blood for analysis. In the embodiment wherein a bridging member is used to provide flow of the test sample across a channel in the membrane, the bridging member may also function as a blood separation filter.

The collection region may be a sub-region of a larger receiving region of the membrane. Prior to supplying the diluent, the collection region having a known saturation volume is separated from the receiving region, for example by scoring or cutting the membrane along a line that defines the collection region.

Various means may be provided to convey the test sample to the collection region. In one embodiment, the test sample may be deposited by the user directly onto the collection region in an amount to ensure saturation of the collection region. In an alternate embodiment, the test sample may be conveyed by structure defined in the membrane, such as a channel. The channel may be disposed along an edge of the collection region for relatively rapid intake of the test sample into the collection region. The test sample may migrate from the channel into the collection region and, after a sufficient time, the collection region is saturated with the test sample. The channel may be in communication with a collection tip for direct intake of the test sample into the channel. For example, the tip may be formed at an end of the channel and configured for placement adjacent to the skin of a user for receipt of a test sample of blood.

In one embodiment wherein a channel is formed in the membrane for conveying the test sample, the channel need not form a boundary of the collection region. The collection region may be separated from the region of the membrane containing the channel by scoring or cutting the membrane prior to supplying the diluent to the collection region.

Any channel structure defined in the membrane may be formed by a suitable microfabrication technique that renders at least a portion of the channel essentially non-conductive to the test sample. Such a technique may include laser ablation or applying a solvent treatment to the membrane. In this instance, a bridging member may be used to conduct the test sample from the channel to the collection region. The channel may be treated with a hydrophobic agent to promote rapid collection of the test sample within the channel.

The invention also includes any manner of test kit utilizing a membrane configured for practicing the method described herein.

Other features and aspects of the present invention are discussed in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

A full and enabling disclosure of the present invention, including the best mode thereof, directed to one of ordinary skill in the art, is set forth more particularly in the remainder of the specification, which makes reference to the appended figures in which:

FIG. **1** is a perspective view of one embodiment of a lateral flow assay device of the present invention.

FIG. **2** is cut-away view of the lateral flow assay device shown in FIG. **1**, particularly illustrating a soluble composition barrier between the collection region and detection region of the membrane.

FIG. **3** is a perspective view of an alternate embodiment of a lateral flow assay device in accordance with aspects of the invention.

FIG. **4** is a cut-away view of the lateral flow assay device shown in FIG. **3**.

FIG. **5** is a top perspective view of a portion of the lateral flow assay device of FIG. **3** particularly illustrating use of bridging member between the collection region and detection region.

FIG. **6** is a perspective view of another embodiment of a lateral flow assay device of the present invention.

Repeat use of reference characters in the present specification and drawings is intended to represent same or analogous features or elements of the invention.

DETAILED DESCRIPTION OF REPRESENTATIVE EMBODIMENTS

Definitions

As used herein, the term "analyte" generally refers to a substance to be detected. For instance, analytes may include antigenic substances, haptens, antibodies, and combinations thereof. Analytes include, but are not limited to, toxins, organic compounds, proteins, peptides, microorganisms, amino acids, nucleic acids, hormones, steroids, vitamins, drugs (including those administered for therapeutic purposes as well as those administered for illicit purposes), drug intermediaries or byproducts, bacteria, virus particles and metabolites of or antibodies to any of the above substances. Specific examples of some analytes include ferritin; creatinine kinase MB (CK-MB); digoxin; phenytoin; phenobarbitol; carbamazepine; vancomycin; gentamycin; theophylline; valproic acid; quinidine; luteinizing hormone (LH); follicle stimulating hormone (FSH); estradiol, progesterone; C-reactive protein; lipocalins; IgE antibodies; cytokines; vitamin B2 micro-globulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B virus surface antigen (HBsAg); antibodies to hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV 1 and 2); human T-cell leukemia virus 1 and 2 (HTLV); hepatitis Be antigen (HBeAg); antibodies to hepatitis Be antigen (Anti-HBe); influenza virus; thyroid stimulating hormone (TSH); thyroxine (T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryoic antigen (CEA); lipoproteins, cholesterol, and triglycerides; and alpha fetoprotein (AFP). Drugs of abuse and controlled substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates, such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines, such as librium and valium; cannabinoids, such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates, such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone and opium; phencyclidine; and propoxyhene. Other potential analytes may be described in U.S. Pat. No. 6,436,651 to Everhart, et al. and U.S. Pat. No. 4,366,241 to Tom et al.

As used herein, the term "test sample" generally refers to a biological material suspected of containing the analyte. The test sample may be derived from any biological source, such as a physiological fluid, including, blood, interstitial fluid, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, mucous, nasal fluid, sputum, synovial fluid, peritoneal fluid, vaginal fluid, menses, amniotic fluid, semen, and so forth. Besides physiological fluids, other liquid samples may be used such as water, food products, and so forth, for the performance of environmental or food production assays. In addition, a solid material suspected of containing the analyte may be used as the test sample. The test sample may be used directly as obtained from the biological source or following a pretreatment to modify the character of the sample. For example, such pretreatment may include preparing plasma from blood, diluting viscous fluids, and so forth. Methods of pretreatment may also involve filtration, precipitation, dilution, distillation, mixing, concentration, inactivation of interfering components, the addition of reagents, lysing, etc. Moreover, it may also be beneficial to modify a solid test sample to form a liquid medium or to release the analyte.

DETAILED DESCRIPTION

Reference now will be made in detail to various embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations may be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, may be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents.

The present invention is directed to a diagnostic method and associated test kit for detecting an analyte residing in a test sample. The method employs a lateral flow device that contains a membrane. The membrane includes a collection region having a known saturation volume for the sample to be tested, and a detection region. A temporary or removable barrier is defined between the collection region and the detection region. In use, the collection region is saturated with a test sample having a volume of less than about 100 microliters so that a known volume of the test sample is contained in the collection region. The barrier is subsequently removed from between the collection region and detection region of the membrane, and a diluent is supplied to the collection region to facilitate flow of the test sample from the collection region to the detection region.

The membrane with defined collection region is particularly well suited for delivering a controlled volume of the test sample to the detection region, and is particularly effective for embodiments in which the test sample has a relatively low volume, such as less than about 100 microliters, in some embodiments less than about 25 microliters, and in some embodiments, less than about 10 microliters. For example, whole blood drops obtained from patients with a lancet from low-pain areas having reduced nerve endings as compared to a fingertip, such as the forearm, thigh, or other alternate sites, may have a volume of from about 0.1 to about 5 microliters. Despite their low volume, the present inventors have discovered that the blood drops may still be accurately analyzed for the presence of an analyte using lateral flow detection techniques.

Referring to FIG. **1**, for instance, one embodiment of a diagnostic test kit that may be formed according to the present invention will now be described in more detail. As

shown, the diagnostic test kit includes a lateral flow assay device **20**, which contains a membrane **23** optionally supported by a rigid support material **21**. In general, the membrane **23** may be made from any of a variety of materials through which the test sample is capable of passing. For example, the membrane **23** may be formed from natural, synthetic, or naturally occurring materials that are synthetically modified, such as polysaccharides (e.g., cellulose materials such as paper and cellulose derivatives, such as cellulose acetate and nitrocellulose); polyether sulfone; polyethylene; nylon; polyvinylidene fluoride (PVDF); polyester; polypropylene; silica; inorganic materials, such as deactivated alumina, diatomaceous earth, $MgSO_4$, or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon or rayon); porous gels, such as silica gel, agarose, dextran, and gelatin; polymeric films, such as polyacrylamide; and so forth. Particularly desired materials for forming the membrane **23** include polymeric materials, such as nitrocellulose, polyether sulfone, polyethylene, nylon, polyvinylidene fluoride, polyester, and polypropylene. It should be understood that the term "nitrocellulose" refers to nitric acid esters of cellulose, which may be nitrocellulose alone, or a mixed ester of nitric acid and other acids, such as aliphatic carboxylic acids having from 1 to 7 carbon atoms.

The size and shape of the membrane **23** may generally vary as is readily recognized by those skilled in the art. For instance, a membrane strip may have a length of from about 10 to about 100 millimeters, in some embodiments from about 20 to about 80 millimeters, and in some embodiments, from about 40 to about 60 millimeters. The width of the membrane strip may also range from about 0.5 to about 20 millimeters, in some embodiments from about 1 to about 15 millimeters, and in some embodiments, from about 2 to about 10 millimeters. Although not required, the thickness of the membrane strip may be small enough to allow transmission-based detection. For example, the membrane strip may have a thickness less than about 500 micrometers, in some embodiments less than about 250 micrometers, and in some embodiments, less than about 150 micrometers.

As stated above, the support **21** carries the membrane **23**. For example, the support **21** may be positioned directly adjacent to the membrane **23** as shown in FIG. **1**, or one or more intervening layers may be positioned between the membrane **23** and the support **21**. Regardless, the support **21** may generally be formed from any material able to carry the membrane **23**. The support **21** may be formed from a material that is transmissive to light, such as transparent or optically diffuse (e.g., transluscent) materials. Also, it is generally desired that the support **21** is liquid-impermeable so that fluid flowing through the membrane **23** does not leak through the support **21**. Examples of suitable materials for the support include, but are not limited to, glass; polymeric materials, such as polystyrene, polypropylene, polyester (e.g., Mylar® film), polybutadiene, polyvinylchloride, polyamide, polycarbonate, epoxides, methacrylates, and polymelamine; and so forth.

To provide a sufficient structural backing for the membrane **23**, the support **21** is generally selected to have a certain minimum thickness. Likewise, the thickness of the support **21** is typically not so large as to adversely affect its optical properties. Thus, for example, the support **21** may have a thickness that ranges from about 100 to about 5,000 micrometers, in some embodiments from about 150 to about 2,000 micrometers, and in some embodiments, from about 250 to about 1,000 micrometers. For instance, one suitable membrane strip having a thickness of about 125 micrometers may be obtained from Millipore Corp. of Bedford, Mass. under the name "SHF180UB25."

As is well known the art, the membrane **23** may be cast onto the support **21**, wherein the resulting laminate may be die-cut to the desired size and shape. Alternatively, the membrane **23** may simply be laminated to the support **21** with, for example, an adhesive. In some embodiments, a nitrocellulose or nylon membrane is adhered to a Mylar® film. An adhesive is used to bind the membrane to the Mylar® film, such as a pressure-sensitive adhesive. Laminate structures of this type are believed to be commercially available from Millipore Corp. of Bedford, Mass. Still other examples of suitable laminate assay device structures are described in U.S. Pat. No. 5,075,077 to Durley, III, et al., which is incorporated herein in its entirety by reference thereto for all purposes.

The device **20** may also contain an absorbent pad (not shown). For example, the absorbent pad may be positioned adjacent to or near an end **27** of the membrane **23**. The absorbent pad generally receives fluid that has migrated through the entire membrane **23**. The absorbent pad may assist in promoting capillary action and fluid flow through the membrane **23**.

The test membrane **23** includes a collection region **68**, which is a portion of the membrane having precisely defined dimensions. The collection region **68** collects and stores the test sample before the sample is conducted to a detection region **31**. Upon saturation of the collection region **68**, a precisely metered volume of the test sample is thus obtained. The correlation of amount of test sample at a saturation condition of a given volume of the collection region **68** may be empirically determined for various test samples and membrane materials and plotted or otherwise stored in a convenient format.

Referring to FIG. **1**, the collection region **68** is separated from the detection region **31** by a barrier **62**. This barrier **62** serves to isolate the collection region **68** and prevent migration of the test sample to the detection region **31** until the collection region **68** has been saturated with the test sample. Once the collection region **68** has been saturated (and the required volume of test sample is thus obtained) the barrier **62** is effectively "removed" so that the test sample can be conducted from the collection region **68** to the detection region **31** by application of a diluent to the collection region **68**.

The barrier **62** may take on various forms. For example, in the embodiment illustrated in FIGS. **1**, **2**, and **6**, the barrier **62** is defined by a soluble composition **64** that is applied in a defined band or stripe across the width of the membrane **23**. This composition **64** permeates through the membrane **23** to the support **21** to effectively define a "dam" across the membrane **23**. The soluble composition **64** must thus have a viscosity to permeate through the membrane **23** and be carefully applied in a well defined band across the membrane **23** so as not to compromise the leading edge dimension of the collection region **68**.

In a desirable embodiment, the soluble composition **64** is dissolvable by the diluent that is used to wash the test sample from the collection region **68** to the detection region **31**. The soluble composition is selected so as not to interfere with or mask the analytical test conducted on the test sample at the detection region **31**. In particular embodiments, the soluble composition may be, for example, sugar, glycerol, and salts.

In other embodiments, for example as illustrated in FIGS. **3**, **4**, and **5**, the barrier **62** is defined by physical structure that essentially separates the collection region **68** from the downstream region of the membrane **23** containing the detection region **31**. This structure may be, for example, a separation or divide **66** in the membrane **23** that spans the width of the membrane. This divide **66** prevents the test sample that has accumulated in the collection region **68** from migrating to the detection region **31**. To initiate flow of the test sample from the collection region **68** to the detection zone **31**, a variety of techniques may be employed. For example, referring to FIG. **5**, a bridging member **51** is shown that is placed over the divide **66** so that it is in fluid communication with the membrane **23**. More specifically, the bridging member **51** has a first end **53** that is contiguous and in fluid communication with the membrane **23** at a location nearer to the detection zone **31** and a second opposing end **55** that is also contiguous and in fluid communication with the collection region **68** of the membrane **23**. The bridging member **51** provides a capillary "lift" that pulls the test sample volume and diluent from the collection region **68**. Once absorbed by the bridging member **51**, the test sample is capable of flowing along the bridging member **51** and through the membrane **23** to the detection zone **31** for analysis. The bridging member **51** may be formed from any material through which the test sample is capable of flowing. For example, the bridging member **51** may be formed from any of the membrane-based materials described above for use in forming the membrane **23**. Some specific materials that may be used include, but are not limited to, nylon, nitrocellulose, cellulose, porous polyethylene pads, and glass fiber filter paper.

When blood is the test sample, the bridging member **51** may also serve the function of a blood separation filter. Blood separation filters selectively retain cellular components (e.g., red blood cells) contained within a whole blood sample and deliver the remaining components of the blood sample (e.g., plasma or serum) to the detection zone. The blood separation filter may be made of any suitable material, for example, a hydrophobic material capable of filtering cells (e.g., blood cells) from fluids. Various packings or sieving depth filters may be employed, such as glass fibers, cellulose or glass filters treated with red blood cell capture reagents, glass fiber filters, synthetic fiber filters or a composite material including any combination of the above materials. Glass fiber filters, for instance, are commercially available from Whatman plc of Kent, United Kingdom; Millipore Corp. of Billerica, Mass.; and Pall Corp. of Ann Arbor, Mich. Such glass fiber filters may have a fiber diameter in the range of about 0.05 to about 9 micrometers and a density of about 50 to about 150 g/m^2. Other examples of suitable blood separation filters are described in U.S. Pat. No. 5,416,000 to Allen, et al., as well as U.S. Patent Application Publication Nos. 2004/0126833 to Shull, et al. and 2003/0032196 to Zhou, all of which are incorporated herein in their entirety by reference thereto for all purposes. If desired, the blood separation filter may be treated with one or more reagents (e.g., agglutinin), such as described above.

The test sample may be applied to the collection region **68** by various means. For example, the user may deposit the test sample directly onto the collection region **68** by a pipette, dropper, or other device. In alternate embodiments, the test sample may be initially received in a channel structure defined in the membrane **23** that aids in rapid intake of the sample and uniform distribution of the sample across the width of the collection region **68**. For example, referring again to FIG. **1**, the lateral flow device **20** may include a channel **35** that is formed in a surface of the membrane **23** upstream of the collection region **68**. Although illustrated as having a rectangular shape, the channel **35** may generally have any desired cross-sectional shape, such as circular, square, triangular, trapezoidal, v-shaped, u-shaped, hexagonal, octagonal, irregular, and so forth. Further, the channel **35** may be straight, tapered, curved, serpentine, labyrinth-like, or have any other desired configuration.

Regardless of the shape selected, the dimensions of the channel **35** are generally such that it is capable of rapidly taking up the test sample via passive capillary flow. Capillary flow generally occurs when the adhesive forces of a fluid to the walls of a channel are greater than the cohesive forces between the liquid molecules. Specifically, capillary pressure is inversely proportional to the cross-sectional dimension of the channel and directly proportional to the surface tension of the liquid, multiplied by the cosine of the contact angle of the fluid in contact with the material forming the channel. Thus, to facilitate capillary flow, the length of the channel **35** in the longitudinal direction "L" of the membrane **23** may be less than about 20 millimeters, in some embodiments from about 0.001 to about 10 millimeters, and in some embodiments, from about 0.01 to about 4 millimeters. Of course, the length may also vary as a function of width.

The dimensions of the channel **35** dictate the volume of the test sample that will be delivered to the collection zone **68**. More specifically, the test sample will quickly fill the empty volume of the channel **35** upon application thereto, thereby controlling the amount of sample delivered to the device **20**. In this regard, the channel **35** should have dimensions to ensure that a sufficient volume of the sample is present for complete saturation of the collection region **68**. To facilitate the delivery of a controlled volume of the test sample to the collection zone **31**, the height or depth of the channel **35** may be varied to accommodate the desired volume of the test sample. For example, the depth of the channel **35** may be from about 0.1 micrometers to about 800 micrometers, in some embodiments from about 20 micrometers to about 400 micrometers, and in some embodiments, from about 80 micrometers to about 200 micrometers. The channel **35** may also have a width (in a direction "W") that is the same or substantially equal to the width of the membrane **23**. In this manner, the test sample will flow more uniformly across the entire width of the collection region **68** upon initiation of the assay. In turn, the test sample will ultimately reach the detection zone **31** in a more uniform manner, thereby providing more accurate results. In some embodiments, for instance, the width of the metering channel **35** ranges from about 0.5 to about 20 millimeters, in some embodiments from about 1 to about 15 millimeters, and in some embodiments, from about 2 to about 10 millimeters. Of course, the width, depth, and/or length of the channel **35** may also vary as a function of the dimension. In such cases, the specified width, depth, or length is an average dimension.

The ability of the channel **35** to take up an aqueous sample (e.g., blood) by capillary action is improved when its surface tension is near or exceeds the surface tension of water (i.e., 72 mN/m). Thus, if desired, the metering channel **35** may be treated with one or more wetting agents to increase surface tension. One type of wetting agent that may be used in the present invention is a hydrophilic wetting agent, such as a nonionic surfactant. Examples of suitable nonionic surfactants include ethoxylated alkylphenols, ethoxylated and propoxylated fatty alcohols, ethylene oxide-propylene oxide block copolymers, ethoxylated esters of fatty (C_8-C_{18}) acids,

condensation products of ethylene oxide with long chain amines or amides, condensation products of ethylene oxide with alcohols, acetylenic diols, and mixtures thereof. Various specific examples of suitable nonionic surfactants include, but are not limited to, methyl gluceth-10, PEG-20 methyl glucose distearate, PEG-20 methyl glucose sesquistearate, C_{11-15} pareth-20, ceteth-8, ceteth-12, dodoxynol-12, laureth-15, PEG-20 castor oil, polysorbate 20, steareth-20, polyoxyethylene-10 cetyl ether, polyoxyethylene-10 stearyl ether, polyoxyethylene-20 cetyl ether, polyoxyethylene-10 oleyl ether, polyoxyethylene-20 oleyl ether, an ethoxylated nonylphenol, ethoxylated octylphenol, ethoxylated dodecylphenol, or ethoxylated fatty (C_6-C_{22}) alcohol, including 3 to 20 ethylene oxide moieties, polyoxyethylene-20 isohexadecyl ether, polyoxyethylene-23 glycerol laurate, polyoxyethylene-20 glyceryl stearate, PPG-10 methyl glucose ether, PPG-20 methyl glucose ether, polyoxyethylene-20 sorbitan monoesters, polyoxyethylene-80 castor oil, polyoxyethylene-15 tridecyl ether, polyoxyethylene-6 tridecyl ether, laureth-2, laureth-3, laureth-4, PEG-3 castor oil, PEG 600 dioleate, PEG 400 dioleate, and mixtures thereof. Commercially available nonionic surfactants may include the SURFYNOL® range of acetylenic diol surfactants available from Air Products and Chemicals of Allentown, Pa.; the TWEEN® range of polyoxyethylene surfactants available from Fisher Scientific of Pittsburgh, Pa.; and the TRITON® range of polyoxyethylene surfactants (e.g., TRITON® X-100, polyoxyethylene-10 isooctylcyclohexyl ether) available from Sigma-Aldrich Chemical Co. of St. Louis, Mo.

Besides surfactants, still other suitable wetting agents may include water-soluble or water-swellable polymers that are substantially more lubricious when wetted with water, or with a water or alcohol-based electrolyte, than when dry. Examples of such hydrophilic polymers include, for instance, sodium, potassium and calcium alginates, carboxymethylcellulose, agar, gelatin, polyvinyl alcohol, collagen, pectin, chitin, chitosan, poly(α-amino acids), polyester, poly-1-caprolactone, polyvinylpyrrolidone, polyethylene oxide, polyvinyl alcohol, polyether, polysaccharide, hydrophilic polyurethane, polyhydroxyacrylate, polymethacrylate, dextran, xanthan, hydroxypropyl cellulose, methyl cellulose, and homopolymers and copolymers of N-vinylpyrrolidone, N-vinyllactam, N-vinyl butyrolactam, N-vinyl caprolactam, other vinyl compounds having polar pendant groups, acrylate and methacrylate having hydrophilic esterifying groups, hydroxyacrylate, acrylic acid, and combinations thereof.

Referring again to FIG. **1**, a score or cut line **70** is indicated by a dashed line. This line may be desired to precisely define the dimensions of the collection region **68** once the entire region of the membrane **23** between the channel **35** and barrier **62** has been saturated with the test sample. This region may be considered as a "receiving region", with the collection region **68** being a sub-part of the receiving region that is subsequently defined along the line **70**. Prior to supplying the diluent to the collection region **68**, it may be necessary to separate the collection region **68** from the channel **35**, for example by cutting or scoring the membrane **23** along the line **70**. This prevents any excess test sample within the channel **35** from being conveyed to the detection region **31**, which would alter the defined volume of the test sample conveyed from the collection region **68**.

The channel **35** (and divide **66**) may generally be formed using any of a variety of different techniques. For example, the channel **35** may be formed by simply laminating separate portions of a membrane onto a support material so that a channel is formed therebetween. As a result, the walls of the metering channel **35** are at least partially formed by respective membrane structures. The wall **38** of the channel **35** may be conductive to the test sample across the width of the channel **35** so that the test sample migrates into the "receiving" region of the membrane. As discussed above, the collection region **68** may be separated from the receiving region along line **70**. In alternate embodiments, diffusion of the test sample from the channel **35** may be inhibited by treating one or both of the walls with a hydrophobic material (e.g., polymer). For example, referring to the embodiments of FIGS. **3** and **4**, the walls **38** and **39** of the channel **35** are rendered non-conductive to the test sample (illustrated by the more dense region along the walls **38** and **39**) to prevent the test sample from migrating from the channel **35** into the collection region **68**. In this case, a bridging member (such as member **51** discussed above) may be used to conduct the test sample from the channel **35** and into the collection region **68**. The channel **35** has a bottom surface defined by the support **21**, which may be formed from a material that is hydrophobic.

In the embodiment of FIGS. **3** and **4**, the non-conductive wall **38** of the channel **35** prevents excess test sample from migrating into the collection region **68** (after removal of the bridging member **51**) and thus may serve to adequately define the edge of the collection region **68** such that it is not necessary to separate the collection region **68** along a line **70**.

The channel **35** (and divide **66**) may be microfabricated into the membrane **23**. Such a microfabrication technique employs only a confined region of the membrane material for channel formation without adversely affecting the remaining portions. Various mechanical microfabrication techniques may be used to accomplish such channel formation, and include, for instance, cutting, laser ablation, photolithography, and so forth. For example, in one particular embodiment of the present invention, laser ablation techniques are used to form the metering channel **35**. Laser ablation generally refers to a process for removing a material using incident light of a certain wavelength. In polymeric materials, for instance, the incident light generally induces photochemical changes in the polymer that results in chemical dissolution. Any known laser may be employed in the present invention, including, for instance, CO_2, pulsed light lasers, diode lasers, ND Yag 1064 nm & 532 nm lasers, Alexandrite and Q-switched lasers, pulsed dye lasers, optical and RF lasers, erbium lasers, ruby lasers, and holmium lasers. For example, a CO_2 laser may be used to etch a nitrocellulose membrane that is mounted on a supporting fixture. Through use of a moving beam or an X-Y table, precision channels may be generated on the nitrocellulose. In addition, various other known optical devices may be employed in conjunction with the laser to enhance the channel formation, such as optical lenses, mirrors, etc. The parameters of the laser ablation technique, such as wavelength, pulse duration, pulse repetition rate, and beam quality, may be selected for forming the channel **35** as is well known to those skilled in the art.

Chemical microfabrication techniques may also be employed in the present invention to form the channel **35** (and divide **66**). For example, a solvent treatment may be employed in the present invention that exhibits a dissolving capacity for the membrane **23**. To ensure that dissolution of the membrane **23** remains confined within the regions of the channel **35**, the dissolving capacity (solvency) of the solvent treatment is generally optimized so that it may quickly form the channel **35** before flowing to other regions of the

membrane **23**. Some examples of suitable solvents that may be used in the solvent treatment include glycols, such as propylene glycol, butylene glycol, triethylene glycol, hexylene glycol, polyethylene glycols, ethoxydiglycol, and dipropyleneglycol; glycol ethers, such as methyl glycol ether, ethyl glycol ether, and isopropyl glycol ether; ethers, such as diethyl ether and tetrahydrofuran; alcohols, such as methanol, ethanol, n-propanol, iso-propanol, and butanol; triglycerides; ketones, such as acetone, methyl ethyl ketone, and methyl isobutyl ketone; esters, such as ethyl acetate, butyl acetate, and methoxypropyl acetate; amides, such as dimethylformamide, dimethylacetamide, dimethylcaprylic/capric fatty acid amide and N-alkylpyrrolidones; nitrites, such as acetonitrile, propionitrile, butyronitrile and benzonitrile; sulfoxides and sulfones, such as dimethyl sulfoxide (DMSO) and sulfolane; and so forth.

Of course, the selected solvent will vary depending on the material used to form the membrane **23**. In one particular embodiment, for example, the membrane **23** is formed from nitrocellulose. Examples of solvents that are capable of dissolving nitrocellulose (i.e., active solvents) include ketones, such as acetone, methyl ethyl ketone, and methyl isobutyl ketone; esters, such as ethyl acetate, butyl acetate, and methoxy propyl acetate; glycol ethers, such as methyl glycol ether, ethyl glycol ether, and isopropyl glycol ether; and alcohols, such as methanol and ethanol. In some embodiments, a latent solvent may be employed that is only capable of dissolving nitrocellulose under certain conditions, such as at a higher temperature or in the presence of an active solvent. Examples of such latent solvents may include, for instance, ethanol, isopropanol, and butanol. In some cases, a mixture of an active solvent and a co-solvent (e.g., latent solvent or other active solvent) may be employed. Such co-solvents may provide synergistic improvement to the dissolving capacity of the active solvent, or may simply be employed to reduce costs. When utilized, the active solvent is typically present in an amount greater than about 50 vol. %, in some embodiments greater than about 60 vol. %, and in some embodiments, from about 70 vol. % to about 95 vol. %. Likewise, the co-solvent may be present in an amount less than about 50 vol. %, in some embodiments less than about 40 wt. %, and in some embodiments, from about 5 vol. % to about 30 vol. %. In still other embodiments, a mixture of two or more latent solvents may be employed.

The purity of a solvent may also influence its dissolving capacity. That is, higher solvent purities generally result in a higher dissolving capacity. Thus, to optimize dissolving capacity, it is normally desired that the purity of the solvent likewise be optimized. For example, in most embodiments, the purity of a solvent employed in the present invention is greater than about 95 mass %, in some embodiments greater than about 98 mass %, and in some embodiments, greater than about 99 mass %.

The solvent treatment may be applied to the membrane using any of a variety of well-known application techniques. Suitable application techniques include, for example, spraying, printing (e.g., inkjet, pad, etc.), pipette, air brushing, metering with a dispensing pump, and so forth. In one particular embodiment, for example, the solvent treatment is applied using a dispensing and optional drying process commonly employed to form detection lines on lateral flow strips. Such a system could involve placing a sheet of the porous membrane on a dispensing machine and threading it through a rewind spindle. This may be accomplished using either a batch or continuous process. The dispensing machine delivers a precise volume of the solvent treatment in a straight line as the membrane passes beneath. The sheet then passes through a drier and is wound back on a spool for further processing. One such lab-scale dispensing pump system for batch processes is available from Kinematic Automation, Inc. of Twain Harte, Calif. under the name "Matrix™ 1600."

The solvent treatment may also be applied in any amount effective to form the channel **35** with the desired size and shape. The ultimate amount employed may depend on a variety of factors, including the dissolving capacity of the solvent for the membrane **23**, the speed of application, etc. For example, in some embodiments, the solvent treatment is applied in an amount of from about 0.01 to about 10 microliters per centimeter in width of the membrane, in some embodiments from about 0.1 to about 10 microliters per centimeter in width of the membrane, and in some embodiments, from about 0.5 to about 5 microliters per centimeter in width of the membrane **23**.

One benefit of the above-described microfabrication techniques is that they may impart barrier properties to the walls of the channel **35** and divide **66** without requiring separate treatment, such as with a hydrophobic material. For example, referring to FIG. **4**, one embodiment of a channel **35** and divide **66** are shown in cross-section that have been subjected to a microfabrication technique. Although the membrane **23** of this embodiment contains pores **60**, any pores previously located near the walls of the channel **35** and divide **66** are either destroyed or substantially reduced in size subsequent to the microfabrication technique. Likewise, the channel **35** and divide **66** have a bottom surface **41** defined by the support **21**, which is generally formed from a material that is hydrophobic. In this manner, flow of the test sample through the walls and beneath the membrane **23** is substantially inhibited. Alternatively, the channel **35** and divide **66** may not extend to the support **21** so that the bottom surface of the structures is formed by the membrane **23**. In such cases, the microfabrication technique may also destroy any pores previously located near the bottom surface.

Regardless of the manner in which it is formed, the channel **35** acts as a mechanism for collecting the test sample until initiation of the assay is desired. The test sample may be applied directly to the metering channel **35** by a user, or it may be supplied to the metering channel **35** from some other location of the assay device **20**, such as from a sample pad, a blood filter, etc. In one embodiment, a user may simply apply a drop of whole blood (e.g., drop obtained from a lancet, a finger, or other alternate site, such as a forearm) to the channel **35**.

If desired, the assay device **20** may be configured to facilitate application of the test sample to the channel **35**. Referring to FIG. **6**, for instance, one embodiment of the assay device **20** is shown that includes a channel **35**, a membrane **23**, and a support **21**. In this particular embodiment, portions of the membrane **23** and support **21** are removed so that a tip **24** is formed at the channel **35**. For example, the tip **24** may provide a location against which a user's skin may be depressed, thereby transferring blood to the metering channel **35**.

When the test sample is whole blood, the metering channel **35** may be treated with a red blood cell agglutinating reagent (i.e., agglutinin) to facilitate separation of the red blood cells from the blood serum. For example, the walls **38**, **39** and/or surface **41** of the channel **35** may be pretreated with such an agglutinating agent. In this manner, only the blood serum or plasma is analyzed at the detection zone **31**, which may enhance the semi-quantitative or quantitative detection of low volume test samples. Agglutinin may be a

lectin, such as concanavalin A or Lycopersicon esculentum, or an antibody that specifically binds erythrocytes, such as a polyclonal rabbit anti-human erythrocyte antibody preparation. Agglutinins are typically applied in an amount sufficient to agglutinate most of the erythrocytes in the test sample. Other reagents may also be applied to selectively bind or retard movement of certain other biological sample constituents. For example, the metering channel **35** may be treated with a reagent that separates red blood cells from plasma so that plasma components, such as an analyte (e.g., C-reactive protein), may be analyzed. Alternatively, a reagent may be applied that selectively separates biological sample components by their biological, chemical, or physical properties. Other reagents that reduce non-specific binding or non-specific adsorption of components of the blood sample may be used to treat the channel **35**. For instance, the channel **35** may be treated with a protein, such as albumin (e.g., bovine serum albumin).

To further aid in transfer of the test sample from the channel **35** to the collection region **68**, an absorbent member may be placed into the channel **35** to absorb the test sample. The bridging member **51** is then placed contiguous and in fluid communication with the absorbent member. In this manner, the test sample may simply flow from the absorbent member to the bridging member **51**.

Regardless of the particular mechanism or method used to saturate the collection region **68** with the test sample, a diluent (or washing agent) is generally employed to facilitate delivery of the test sample from the collection region **68** to the detection zone **31**. The diluent is typically applied upstream from the collection region **68** so that it may initiate flow in the direction of the detection zone **31**. For example, in the embodiment of FIG. **5**, upon application, the diluent will mix with the test sample and flow through the membrane **23** of the collection region **68** until reaching the first end **53** of the bridging member **51**. The diluent and test sample will then flow through the bridging member **51** to the second end **55** of the bridging member **51**. Finally, the diluent/test sample mixture flows from the second end **55** to the detection zone **31** for analysis.

In the embodiment of FIGS. **1** and **2**, diluent/test sample mixture flows through the membrane **23** of the collection region **68** until encountering the soluble composition **64**. The diluent will dissolve the composition **64** and the mixture is free to flow into the detection zone **31**.

The diluent may be any material having a viscosity that is sufficiently low to allow movement of the fluid by capillary action and that supports a reaction between the analyte and any binding agents (e.g., does not interfere with antibody/antigen interaction). In one embodiment, the diluent contains water, a buffering agent; a salt (e.g., NaCl); a protein stabilizer (e.g., BSA, casein, trehalose, or serum); and/or a detergent (e.g., nonionic surfactant). Representative buffering agents include, for example, phosphate-buffered saline (PBS) (e.g., pH of 7.2), 2-(N-morpholino) ethane sulfonic acid (MES) (e.g., pH of 5.3), HEPES buffer, TBS buffer, etc., and so forth.

In addition to the components set forth above, the diagnostic test kit of the present invention may also contain various other components to enhance detection accuracy. For exemplary purposes only, one embodiment of an immunoassay that may be performed in accordance with the present invention to detect the presence will now be described in more detail. Immunoassays utilize mechanisms of the immune systems, wherein antibodies are produced in response to the presence of antigens that are pathogenic or foreign to the organisms. These antibodies and antigens, i.e., immunoreactants, are capable of binding with one another, thereby causing a highly specific reaction mechanism that may be used to determine the presence or concentration of that particular antigen in a biological sample.

To initiate the immunoassay, the test sample (e.g., whole blood) is initially applied to the channel **35** or directly onto the collection region **68**, such as with a lancet, needle, dropper, pipette, capillary device, etc. Once the collection region **68** is saturated with the test sample such that a known desired volume of the test sample is contained within the collection region **68**, the bridging member **51** is placed over the divide **66** in the embodiment of FIGS. **4** and **5** and the diluent is applied to the device **20**. The application of the bridging member **51** and diluent may occur simultaneously or sequentially, and may be performed by manual or automatic operation. The location of diluent application may vary as desired. For example, in some embodiments, the diluent is applied to an additional membrane, such as a sample pad (not shown) or conjugate pad (not shown), which are in fluid communication with a membrane **23**. The sample pad and conjugate pad may be formed from any material through which a fluid is capable of passing, such as glass fibers. Further, if desired, the channel **35** may be formed in the sample pad and/or conjugate pad in the manner described above.

To facilitate the detection of the analyte within the test sample, a substance may be pre-applied to the sample pad and/or conjugate pad, or previously mixed with the diluent or test sample, which is detectable either visually or by an instrumental device. Any substance generally capable of producing a signal that is detectable visually or by an instrumental device may be used as detection probes. Suitable detectable substances may include, for instance, luminescent compounds (e.g., fluorescent, phosphorescent, etc.); radioactive compounds; visual compounds (e.g., colored dye or metallic substance, such as gold); liposomes or other vesicles containing signal-producing substances; enzymes and/or substrates, and so forth. Other suitable detectable substances may be described in U.S. Pat. No. 5,670,381 to Jou, et al. and U.S. Pat. No. 5,252,459 to Tarcha, et al., which are incorporated herein in their entirety by reference thereto for all purposes. If the detectable substance is colored, the ideal electromagnetic radiation is light of a complementary wavelength. For instance, blue detection probes strongly absorb red light.

In some embodiments, the detectable substance may be a luminescent compound that produces an optically detectable signal. For example, suitable fluorescent molecules may include, but are not limited to, fluorescein, europium chelates, phycobiliprotein, rhodamine, and their derivatives and analogs. Other suitable fluorescent compounds are semiconductor nanocrystals commonly referred to as "quantum dots." For example, such nanocrystals may contain a core of the formula CdX, wherein X is Se, Te, S, and so forth. The nanocrystals may also be passivated with an overlying shell of the formula YZ, wherein Y is Cd or Zn, and Z is S or Se. Other examples of suitable semiconductor nanocrystals may also be described in U.S. Pat. No. 6,261,779 to Barbera-Guillem, et al. and U.S. Pat. No. 6,585,939 to Dapprich, which are incorporated herein in their entirety by reference thereto for all purposes.

Further, suitable phosphorescent compounds may include metal complexes of one or more metals, such as ruthenium, osmium, rhenium, iridium, rhodium, platinum, indium, palladium, molybdenum, technetium, copper, iron, chromium, tungsten, zinc, and so forth. Especially preferred are ruthenium, rhenium, osmium, platinum, and palladium. The

metal complex may contain one or more ligands that facilitate the solubility of the complex in an aqueous or non-aqueous environment. For example, some suitable examples of ligands include, but are not limited to, pyridine; pyrazine; isonicotinamide; imidazole; bipyridine; terpyridine; phenanthroline; dipyridophenazine; porphyrin, porphine, and derivatives thereof. Such ligands may be, for instance, substituted with alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, carboxylate, carboxaldehyde, carboxamide, cyano, amino, hydroxy, imino, hydroxycarbonyl, aminocarbonyl, amidine, guanidinium, ureide, sulfur-containing groups, phosphorus containing groups, and the carboxylate ester of N-hydroxy-succinimide.

Porphyrins and porphine metal complexes possess pyrrole groups coupled together with methylene bridges to form cyclic structures with metal chelating inner cavities. Many of these molecules exhibit strong phosphorescence properties at room temperature in suitable solvents (e.g., water) and an oxygen-free environment. Some suitable porphyrin complexes that are capable of exhibiting phosphorescent properties include, but are not limited to, platinum(II) coproporphyrin-I and III, palladium(II) coproporphyrin, ruthenium coproporphyrin, zinc(II)-coproporphyrin-I, derivatives thereof, and so forth. Similarly, some suitable porphine complexes that are capable of exhibiting phosphorescent properties include, but not limited to, platinum(II) tetra-meso-fluorophenylporphine and palladium(II) tetra-meso-fluorophenylporphine. Still other suitable porphyrin and/or porphine complexes are described in U.S. Pat. No. 4,614,723 to Schmidt, et al.; U.S. Pat. No. 5,464,741 to Hendrix; U.S. Pat. No. 5,518,883 to Soini; U.S. Pat. No. 5,922,537 to Ewart, et al.; U.S. Pat. No. 6,004,530 to Sagner, et al.; and U.S. Pat. No. 6,582,930 to Ponomarev, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Bipyridine metal complexes may also be utilized as phosphorescent compounds. Some examples of suitable bipyridine complexes include, but are note limited to, bis [(4,4'-carbomethoxy)-2,2'-bipyridine]2-[3-(4-methyl-2,2'-bipyridine-4-yl)propyl]-1,3-dioxolane ruthenium(II); bis(2,2'bipyridine)[4-(butan-1-al)-4'-methyl-2,2'-bi-pyridine] ruthenium(II); bis(2,2'-bipyridine)[4-(4'-methyl-2,2'-bipyridine-4'-yl)-butyric acid]ruthenium(II); tris(2,2'bipyridine)ruthenium(II); (2,2'-bipyridine)[bis-bis(1,2-diphenylphosphino)ethylene]2-[3-(4-methyl-2,2'-bipyridine-4'-yl)propyl]-1,3-dioxolane osmium(II); bis(2,2'-bipyridine)[4-(4'-methyl-2,2'-bipyridine)-butylamine] ruthenium(II); bis(2,2'-bipyridine)[1-bromo-4(4'-methyl-2,2'-bipyridine-4-yl)butane]ruthenium(II); bis(2,2'-bipyridine)maleimidohexanoic acid, 4-methyl-2,2'-bipyridine-4'-butylamide ruthenium(II), and so forth. Still other suitable metal complexes that may exhibit phosphorescent properties may be described in U.S. Pat. No. 6,613,583 to Richter, et al.; U.S. Pat. No. 6,468,741 to Massey, et al.; U.S. Pat. No. 6,444,423 to Meade, et al.; U.S. Pat. No. 6,362,011 to Massey, et al.; U.S. Pat. No. 5,731,147 to Bard, et al.; and U.S. Pat. No. 5,591,581 to Massey, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

In some cases, luminescent compounds may have a relatively long emission lifetime may have a relatively large "Stokes shift." The term "Stokes shift" is generally defined as the displacement of spectral lines or bands of luminescent radiation to a longer emission wavelength than the excitation lines or bands. A relatively large Stokes shift allows the excitation wavelength of a luminescent compound to remain far apart from its emission wavelengths and is desirable because a large difference between excitation and emission wavelengths makes it easier to eliminate the reflected excitation radiation from the emitted signal. Further, a large Stokes shift also minimizes interference from luminescent molecules in the sample and/or light scattering due to proteins or colloids, which are present with some body fluids (e.g., blood). In addition, a large Stokes shift also minimizes the requirement for expensive, high-precision filters to eliminate background interference. For example, in some embodiments, the luminescent compounds have a Stokes shift of greater than about 50 nanometers, in some embodiments greater than about 100 nanometers, and in some embodiments, from about 100 to about 350 nanometers.

For example, exemplary fluorescent compounds having a large Stokes shift include lanthanide chelates of samarium (Sm(III)), dysprosium (Dy(III)), europium (Eu(III)), and terbium (Tb(III)). Such chelates may exhibit strongly red-shifted, narrow-band, long-lived emission after excitation of the chelate at substantially shorter wavelengths. Typically, the chelate possesses a strong ultraviolet excitation band due to a chromophore located close to the lanthanide in the molecule. Subsequent to excitation by the chromophore, the excitation energy may be transferred from the excited chromophore to the lanthanide. This is followed by a fluorescence emission characteristic of the lanthanide. Europium chelates, for instance, have Stokes shifts of about 250 to about 350 nanometers, as compared to only about 28 nanometers for fluorescein. Also, the fluorescence of europium chelates is long-lived, with lifetimes of about 100 to about 1000 microseconds, as compared to about 1 to about 100 nanoseconds for other fluorescent labels. In addition, these chelates have a narrow emission spectra, typically having bandwidths less than about 10 nanometers at about 50% emission. One suitable europium chelate is N-(p-isothiocyanatobenzyl)-diethylene triamine tetraacetic acid-Eu^{+3}.

In addition, lanthanide chelates that are inert, stable, and intrinsically fluorescent in aqueous solutions or suspensions may also be used in the present invention to negate the need for micelle-forming reagents, which are often used to protect chelates having limited solubility and quenching problems in aqueous solutions or suspensions. One example of such a chelate is 4-[2-(4-isothiocyanatophenyl)ethynyl]-2,6-bis([N,N-bis(carboxymethyl)amino]methyl)-pyridine [Ref: Lovgren, T., et al.; Clin. Chem. 42, 1196-1201 (1996)]. Several lanthanide chelates also show exceptionally high signal-to-noise ratios. For example, one such chelate is a tetradentate β-diketonate-europium chelate [Ref: Yuan, J. and Matsumoto, K.; Anal. Chem. 70, 596-601 (1998)]. In addition to the fluorescent labels described above, other labels that are suitable for use in the present invention may be described in U.S. Pat. No. 6,030,840 to Mullinax, et al.; U.S. Pat. No. 5,585,279 to Davidson; U.S. Pat. No. 5,573,909 to Singer, et al.; U.S. Pat. No. 6,242,268 to Wieder, et al.; and U.S. Pat. No. 5,637,509 to Hemmila, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Detectable substances, such as described above, may be used alone or in conjunction with a particle (sometimes referred to as "beads" or "microbeads"). For instance, naturally occurring particles, such as nuclei, mycoplasma, plasmids, plastids, mammalian cells (e.g., erythrocyte ghosts), unicellular microorganisms (e.g., bacteria), polysaccharides (e.g., agarose), etc., may be used. Further, synthetic particles may also be utilized. For example, in one embodiment, latex microparticles that are labeled with a fluorescent or colored dye are utilized. Although any synthetic particle may be used in the present invention, the particles are typically formed

from polystyrene, butadiene styrenes, styreneacrylic-vinyl terpolymer, polymethylmethacrylate, polyethylmethacrylate, styrene-maleic anhydride copolymer, polyvinyl acetate, polyvinylpyridine, polydivinylbenzene, polybutyleneterephthalate, acrylonitrile, vinylchloride-acrylates, and so forth, or an aldehyde, carboxyl, amino, hydroxyl, or hydrazide derivative thereof. Other suitable particles may be described in U.S. Pat. No. 5,670,381 to Jou, et al.; U.S. Pat. No. 5,252,459 to Tarcha, et al.; and U.S. Patent Publication No. 2003/0139886 to Bodzin, et al., which are incorporated herein in their entirety by reference thereto for all purposes. Commercially available examples of suitable fluorescent particles include fluorescent carboxylated microspheres sold by Molecular Probes, Inc. under the trade names "FluoSphere" (Red 580/605) and "TransfluoSphere" (543/620), as well as "Texas Red" and 5- and 6-carboxytetramethylrhodamine, which are also sold by Molecular Probes, Inc. In addition, commercially available examples of suitable colored, latex microparticles include carboxylated latex beads sold by Bang's Laboratory, Inc. Metallic particles (e.g., gold particles) may also be utilized in the present invention.

When utilized, the shape of the particles may generally vary. In one particular embodiment, for instance, the particles are spherical in shape. However, it should be understood that other shapes are also contemplated by the present invention, such as plates, rods, discs, bars, tubes, irregular shapes, etc. In addition, the size of the particles may also vary. For instance, the average size (e.g., diameter) of the particles may range from about 0.1 nanometers to about 100 microns, in some embodiments, from about 1 nanometer to about 10 microns, and in some embodiments, from about 10 to about 100 nanometers.

In some instances, it may be desired to modify the detection probes in some manner so that they are more readily able to bind to the analyte. In such instances, the detection probes may be modified with certain specific binding members that are adhered thereto to form conjugated probes. Specific binding members generally refer to a member of a specific binding pair, i.e., two different molecules where one of the molecules chemically and/or physically binds to the second molecule. For instance, immunoreactive specific binding members may include antigens, haptens, aptamers, antibodies (primary or secondary), and complexes thereof, including those formed by recombinant DNA methods or peptide synthesis. An antibody may be a monoclonal or polyclonal antibody, a recombinant protein or a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other specific binding members. The details of the preparation of such antibodies and their suitability for use as specific binding members are well known to those skilled in the art. Other common specific binding pairs include but are not limited to, biotin and avidin (or derivatives thereof), biotin and streptavidin, carbohydrates and lectins, complementary nucleotide sequences (including probe and capture nucleic acid sequences used in DNA hybridization assays to detect a target nucleic acid sequence), complementary peptide sequences including those formed by recombinant methods, effector and receptor molecules, hormone and hormone binding protein, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, and so forth. Furthermore, specific binding pairs may include members that are analogs of the original specific binding member. For example, a derivative or fragment of the analyte (i.e., "analog") may be used so long as it has at least one epitope in common with the analyte.

The specific binding members may generally be attached to the detection probes using any of a variety of well-known techniques. For instance, covalent attachment of the specific binding members to the detection probes (e.g., particles) may be accomplished using carboxylic, amino, aldehyde, bromoacetyl, iodoacetyl, thiol, epoxy and other reactive or linking functional groups, as well as residual free radicals and radical cations, through which a protein coupling reaction may be accomplished. A surface functional group may also be incorporated as a functionalized co-monomer because the surface of the detection probe may contain a relatively high surface concentration of polar groups. In addition, although detection probes are often functionalized after synthesis, such as with poly(thiophenol), the detection probes may be capable of direct covalent linking with a protein without the need for further modification. For example, in one embodiment, the first step of conjugation is activation of carboxylic groups on the probe surface using carbodiimide. In the second step, the activated carboxylic acid groups are reacted with an amino group of an antibody to form an amide bond. The activation and/or antibody coupling may occur in a buffer, such as phosphate-buffered saline (PBS) (e.g., pH of 7.2) or 2-(N-morpholino) ethane sulfonic acid (MES) (e.g., pH of 5.3). The resulting detection probes may then be contacted with ethanolamine, for instance, to block any remaining activated sites. Overall, this process forms a conjugated detection probe, where the antibody is covalently attached to the probe. Besides covalent bonding, other attachment techniques, such as physical adsorption, may also be utilized in the present invention.

Referring again to the figures in general, after passing through the collection region **68**, the diluent and test sample travel through the membrane **23** until reaching the detection zone **31**. Upon reaching the detection zone **31**, the volume of the test sample is relatively uniform across the entire width of the detection zone **31**. In addition, as a result of the known saturation volume of the collection region **68**, the volume of the test sample is also predetermined within a narrow range.

Within the detection zone **31**, a receptive material is immobilized that is capable of binding to the conjugated detection probes. The receptive material may be selected from the same materials as the specific binding members described above, including, for instance, antigens; haptens; antibody-binding proteins, such as protein A, protein G, or protein A/G; neutravidin (a deglycosylated avidin derivative), avidin (a highly cationic 66,000-dalton glycoprotein), streptavidin (a nonglycosylated 52,800-dalton protein), or captavidin (a nitrated avidin derivative); primary or secondary antibodies, and derivatives or fragments thereof. In one embodiment, for example, the receptive material is an antibody specific to an antigen within the test sample. The receptive material serves as a stationary binding site for complexes formed between the analyte and the conjugated detection probes. Specifically, analytes, such as antibodies, antigens, etc., typically have two or more binding sites (e.g., epitopes). Upon reaching the detection zone **31**, one of these binding sites is occupied by the specific binding member of the conjugated probe. However, the free binding site of the analyte may bind to the immobilized first receptive material. Upon being bound to the immobilized receptive material, the complexed probes form a new ternary sandwich complex.

Other than the detection zone **31**, the lateral flow device **20** may also define various other zones for enhancing detection accuracy. For example, in embodiments in which high analyte concentrations are a concern, the assay device **20** may contain an indicator zone **33** that is positioned downstream from the detection zone **31** and is configured to

provide information as to whether the analyte concentration has reached the saturation concentration ("hook effect" region) for the assay. The indicator zone **33** contains a second receptive material that is immobilized on the membrane **23** and serves as a stationary binding site for the conjugated detection probes. To accomplish the desired binding within the indicator zone **33**, it is generally desired that the second receptive material is capable of differentiating between those detection probes that are complexed with the analyte and those that remain uncomplexed. For example, in one embodiment, the second receptive material includes a molecule that has at least one epitope in common with the analyte, such as analyte molecules, or derivatives or fragments (i.e., analog) thereof, so that it is capable of specifically binding to an antibody conjugate when it is uncomplexed with the analyte.

Alternatively, the second receptive material may include a biological material that is not an analyte molecule or analog thereof, but nevertheless is capable of preferentially binding to uncomplexed conjugated detection probes. In one embodiment, for example, the first receptive material may be a monoclonal antibody, such as anti-CRP IgG_1. The detection probes are conjugated with a monoclonal antibody different than the monoclonal antibody of the first receptive material, such as anti-CRP IgG_2. In this particular embodiment, the second receptive material may be a secondary antibody, such as Goat anti-human, IgG $F(ab')_2$, which has been adsorbed against F_c fragments and therefore reacts only with the F_{ab} portion of IgG. Thus, when no analyte is present, the secondary antibody is able to bind to the free "Fab" binding domain of the anti-CRP IgG_2 monoclonal antibody. However, when an antigen is present in the test sample, it first complexes with the "Fab" binding domain of the anti-CRP IgG_2 monoclonal antibody. The presence of the antigen renders the "Fab" binding domain unavailable for subsequent binding with the secondary antibody. In this manner, the secondary antibody within the indicator zone **35** is capable of preferentially binding to uncomplexed detection probes.

Although the detection zone **31** and optional indicator zone **33** may provide accurate results, it is sometimes difficult to determine the relative concentration of the analyte within the test sample under actual test conditions. Thus, the assay device **20** may include a calibration zone **32**. In this embodiment, the calibration zone **32** is formed on the membrane **23** and is positioned downstream from the detection zone **31** and optional indicator zone **35**. Alternatively, however, the calibration zone **32** may also be positioned upstream from the detection zone **31** and/or optional indicator zone **33**. The calibration zone **32** is provided with a third receptive material that is capable of binding to any calibration probes that pass through the length of the membrane **23**. When utilized, the calibration probes may contain a detectable substance that is the same or different than the detectable substance used for the detection probes. Moreover, the calibration probes may also be conjugated with a specific binding member, such as described above. For example, in one embodiment, biotinylated calibration probes may be used. Generally speaking, the calibration probes are selected in such a manner that they do not bind to the first or second receptive material at the detection zone **31** and indicator zone **33**. The third receptive material of the calibration zone **32** may be the same or different than the receptive materials used in the detection zone **31** or indicator zone **33**. For example, in one embodiment, the third receptive material is a biological receptive material, such as antigens, haptens, antibody-binding proteins (e.g., protein A, protein G, or protein A/G), neutravidin, avidin, streptavidin, captavidin, primary or secondary antibodies, or complexes thereof. It may also be desired to utilize various non-biological materials for the third receptive material (e.g., polyelectrolytes) of the calibration zone **32**, such as described in U.S. Patent Application Publication No. 2003/0124739 to Song, et al., which is incorporated herein in its entirety by reference thereto for all purposes.

When utilized, the polyelectrolytes may have a net positive or negative charge, as well as a net charge that is generally neutral. For instance, some suitable examples of polyelectrolytes having a net positive charge include, but are not limited to, polylysine (commercially available from Sigma-Aldrich Chemical Co., Inc. of St. Louis, Mo.), polyethyleneimine; epichlorohydrin-functionalized polyamines and/or polyamidoamines, such as poly(dimethylamine-co-epichlorohydrin); polydiallyldimethyl-ammonium chloride; cationic cellulose derivatives, such as cellulose copolymers or cellulose derivatives grafted with a quaternary ammonium water-soluble monomer; and so forth. In one particular embodiment, CelQuat® SC-230M or H-100 (available from National Starch & Chemical, Inc.), which are cellulosic derivatives containing a quaternary ammonium water-soluble monomer, may be utilized. Moreover, some suitable examples of polyelectrolytes having a net negative charge include, but are not limited to, polyacrylic acids, such as poly(ethylene-co-methacrylic acid, sodium salt), and so forth. It should also be understood that other polyelectrolytes may also be utilized, such as amphiphilic polyelectrolytes (i.e., having polar and non-polar portions). For instance, some examples of suitable amphiphilic polyelectrolytes include, but are not limited to, poly(styryl-b-N-methyl 2-vinyl pyridnium iodide) and poly(styryl-b-acrylic acid), both of which are available from Polymer Source, Inc. of Dorval, Canada.

Although any polyelectrolyte may generally be used, the polyelectrolyte selected for a particular application may vary depending on the nature of the detection probes, the calibration probes, the membrane, and so forth. In particular, the distributed charge of a polyelectrolyte allows it to bind to substances having an opposite charge. Thus, for example, polyelectrolytes having a net positive charge are often better equipped to bind with probes that are negatively charged, while polyelectrolytes that have a net negative charge are often better equipped to bind to probes that are positively charged. Thus, in such instances, the ionic interaction between these molecules allows the required binding to occur within the calibration zone **32**. Nevertheless, although ionic interaction is primarily utilized to achieve the desired binding in the calibration zone **32**, polyelectrolytes may also bind with probes having a similar charge.

Because the polyelectrolyte is designed to bind to probes, it is typically desired that the polyelectrolyte be substantially non-diffusively immobilized on the surface of the membrane **23**. Otherwise, the probes would not be readily detectable by a user. Thus, the polyelectrolytes may be applied to the membrane **23** in such a manner that they do not substantially diffuse into the matrix of the membrane **23**. In particular, the polyelectrolytes typically form an ionic and/or covalent bond with functional groups present on the surface of the membrane **23** so that they remain immobilized thereon. Although not required, the formation of covalent bonds between the polyelectrolyte and the membrane **23** may be desired to more permanently immobilize the polyelectrolyte thereon. For example, in one embodiment, the monomers used to form the polyelectrolyte are first formed into a solution and then applied directly to the membrane **23**.

Various solvents (e.g., organic solvents, water, etc.) may be utilized to form the solution. Once applied, the polymerization of the monomers is initiated using heat, electron beam radiation, free radical polymerization, and so forth. In some instances, as the monomers polymerize, they form covalent bonds with certain functional groups of the membrane **23**, thereby immobilizing the resulting polyelectrolyte thereon. For example, in one embodiment, an ethyleneimine monomer may form a covalent bond with a carboxyl group present on the surface of some membranes (e.g., nitrocellulose).

In another embodiment, the polyelectrolyte may be formed prior to application to the membrane **23**. If desired, the polyelectrolyte may first be formed into a solution using organic solvents, water, and so forth. Thereafter, the polyelectrolytic solution is applied directly to the membrane **23** and then dried. Upon drying, the polyelectrolyte may form an ionic bond with certain functional groups present on the surface of the membrane **23** that have a charge opposite to the polyelectrolyte. For example, in one embodiment, positively-charged polyethyleneimine may form an ionic bond with negatively-charged carboxyl groups present on the surface of some membranes (e.g., nitrocellulose).

In addition, the polyelectrolyte may also be crosslinked to the membrane **23** using various well-known techniques. For example, in some embodiments, epichlorohydrin-functionalized polyamines and/or polyamidoamines may be used as a crosslinkable, positively-charged polyelectrolyte. Examples of these materials are described in U.S. Pat. No. 3,700,623 to Keim and U.S. Pat. No. 3,772,076 to Keim, U.S. Pat. No. 4,537,657 to Keim, which are incorporated herein in their entirety by reference thereto for all purposes and are believed to be sold by Hercules, Inc., Wilmington, Del. under the Kymene™ trade designation. For instance, Kymene™ 450 and 2064 are epichlorohydrin-functionalized polyamine and/or polyamidoamine compounds that contain epoxide rings and quaternary ammonium groups that may form covalent bonds with carboxyl groups present on certain types of membranes (e.g., nitrocellulose) and crosslink with the polymer backbone of the membrane when cured. In some embodiments, the crosslinking temperature may range from about 50° C. to about 120° C. and the crosslinking time may range from about 10 to about 600 seconds.

Although various techniques for non-diffusively immobilizing polyelectrolytes on the membrane **23** have been described above, it should be understood that any other technique for non-diffusively immobilizing polyelectrolytic compounds may be used in the present invention. In fact, the aforementioned methods are only intended to be exemplary of the techniques that may be used in the present invention. For example, in some embodiments, certain components may be added to the polyelectrolyte solution that may substantially inhibit the diffusion of such polyelectrolytes into the matrix of the membrane **23**.

The detection zone **31**, indicator zone **33**, and calibration zone **32** may each provide any number of distinct detection regions so that a user may better determine the concentration of one or more analytes within a test sample. Each region may contain the same receptive materials, or may contain different receptive materials. For example, the zones may include two or more distinct regions (e.g., lines, dots, etc.). The regions may be disposed in the form of lines in a direction that is substantially perpendicular to the flow of the test sample through the assay device **20**. Likewise, in some embodiments, the regions may be disposed in the form of lines in a direction that is substantially parallel to the flow of the test sample through the assay device **20**.

In some cases, the membrane **23** may also define a control zone (not shown) that gives a signal to the user that the assay is performing properly. For instance, the control zone (not shown) may contain an immobilized receptive material that is generally capable of forming a chemical and/or physical bond with probes or with the receptive material immobilized on the probes. Some examples of such receptive materials include, but are not limited to, antigens, haptens, antibodies, protein A or G, avidin, streptavidin, secondary antibodies, and complexes thereof. In addition, it may also be desired to utilize various non-biological materials for the control zone receptive material. For instance, in some embodiments, the control zone receptive material may also include a polyelectrolyte, such as described above, that may bind to uncaptured probes. Because the receptive material at the control zone is only specific for probes, a signal forms regardless of whether the analyte is present. The control zone may be positioned at any location along the membrane **23**, but is preferably positioned downstream from the detection zone **31** and the indicator zone **33**.

Qualitative, semi-quantitative, and quantitative results may be obtained in accordance with the present invention. For example, when it is desired to semi-quantitatively or quantitatively detect an analyte, the intensity of any signals produced at the detection zone **31**, indicator zone **33**, and/or calibration zone **32** may be measured with an optical reader. The actual configuration and structure of the optical reader may generally vary as is readily understood by those skilled in the art. For example, optical detection techniques that may be utilized include, but are not limited to, luminescence (e.g., fluorescence, phosphorescence, etc.), absorbance (e.g., fluorescent or non-fluorescent), diffraction, etc. One suitable reflectance spectrophotometer is described, for instance, in U.S. Patent App. Pub. No. 2003/0119202 to Kaylor, et al., which is incorporated herein in its entirety by reference thereto for all purposes. In another embodiment, a reflectance-mode spectrofluorometer may be used to detect the intensity of a fluorescence signal. Suitable spectrofluorometers and related detection techniques are described, for instance, in U.S. Patent App. Pub. No. 2004/0043502 to Song, et al., which is incorporated herein in its entirety by reference thereto for all purposes. Likewise, a transmission-mode detection system may also be used to signal intensity.

Although various embodiments of device configurations have been described above, it should be understood, that a device of the present invention may generally have any configuration desired, and need not contain all of the components described above. Various other device configurations, for instance, are described in U.S. Pat. No. 5,395,754 to Lambotte, et al.; U.S. Pat. No. 5,670,381 to Jou, et al.; and U.S. Pat. No. 6,194,220 to Malick, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

Various assay formats may also be used to test for the presence or absence of an analyte using the assay device of the present invention. For instance, a "sandwich" format typically involves mixing the test sample with detection probes conjugated with a specific binding member (e.g., antibody) for the analyte to form complexes between the analyte and the conjugated probes. These complexes are then allowed to contact a receptive material (e.g., antibodies) immobilized within the detection zone. Binding occurs between the analyte/probe conjugate complexes and the immobilized receptive material, thereby localizing "sandwich" complexes that are detectable to indicate the presence of the analyte. This technique may be used to obtain quantitative or semi-quantitative results. Some examples of

such sandwich-type assays are described by U.S. Pat. No. 4,168,146 to Grubb, et al. and U.S. Pat. No. 4,366,241 to Tom, et al., which are incorporated herein in their entirety by reference thereto for all purposes. In a competitive assay, the labeled probe is generally conjugated with a molecule that is identical to, or an analog of, the analyte. Thus, the labeled probe competes with the analyte of interest for the available receptive material. Competitive assays are typically used for detection of analytes such as haptens, each hapten being monovalent and capable of binding only one antibody molecule. Examples of competitive immunoassay devices are described in U.S. Pat. No. 4,235,601 to Deutsch, et al., U.S. Pat. No. 4,442,204 to Liotta, and U.S. Pat. No. 5,208,535 to Buechler, et al., which are incorporated herein in their entirety by reference thereto for all purposes. Various other device configurations and/or assay formats are also described in U.S. Pat. No. 5,395,754 to Lambotte, et al.; U.S. Pat. No. 5,670,381 to Jou, et al.; and U.S. Pat. No. 6,194,220 to Malick, et al., which are incorporated herein in their entirety by reference thereto for all purposes.

As a result of the present invention, a controlled volume of a test sample may be uniformly delivered to a detection zone of a lateral flow assay device. Such control over sample flow provides a significant improvement in detection accuracy and sensitivity for lateral flow systems. One particular benefit is that sample application and testing may be done in a relatively quick, easy, and simple manner. Further, as a result of the controlled flow provided by the present invention, low volume test samples may be accurately tested without the requirement of complex and expensive equipment to obtain a useable sample. For example, whole blood drops having a volume of less than about 3 microliters may be readily analyzed for the presence of an analyte in accordance with the present invention.

While the invention has been described in detail with respect to the specific embodiments thereof, it will be appreciated that those skilled in the art, upon attaining an understanding of the foregoing, may readily conceive of alterations to, variations of, and equivalents to these embodiments. Accordingly, the scope of the present invention should be assessed as that of the appended claims and any equivalents thereto.

What is claimed is:

1. A diagnostic test kit for detecting the presence of an analyte within a test sample, the test kit comprising a lateral flow assay device that further includes:

a membrane comprising a sample receiving region, a collection region and a detection region, said collection region physically defined so as to have a known saturation volume with respect to said test sample of less than about 100 microliters, such that upon saturation of said collection region, a known volume of the test sample is contained in said collection region; and

a barrier disposed between said collection region and said detection region, wherein said barrier is removed to facilitate flow of said test sample from said collection region to said detection region of said membrane;

said receiving region disposed for initial receipt of the test sample, and further comprising means for physically seperating said collection region into its known saturation volume after receipt of the test sample from said receiving region and prior to supplying a diluent to said collection region.

2. The test kit of claim **1**, further comprising a plurality of detection probes within said detection region that are capable of producing a detectable signal.

3. The test kit of claim **2**, wherein a receptive material is immobilized within said detection region that is capable of binding to said detection probes or conjugates thereof.

4. The test kit of claim **1**, wherein said barrier comprises a soluble composition in a defined band across the width of said membrane, said soluble composition present throughout said membrane in an amount to prevent migration of the test sample from said collection region to said detection region of said membrane.

5. The test kit of claim **4**, wherein said soluble composition is dissolvable by the diluent.

6. The test kit of claim **1**, wherein said barrier comprises a physical separation across said membrane, and further comprising a bridge configured to be placed across said divide so that the test sample flows from said collection region to said detection region with the diluent.

7. The test kit of claim **6**, wherein the test sample is whole blood, said bridge configured as a blood separation filter.

8. The test kit of claim **1**, wherein said collection region comprises a portion of said receiving region and is separable from said receiving region prior to supplying the diluent to said collection region.

9. The test kit of claim **1**, further comprising a channel defined in the membrane to convey the test sample to said collection region.

10. The test kit of claim **9**, further comprising a sampling tip disposed at an end of said channel, said tip configured for placement adjacent to the skin of a user for receipt of a test sample of blood.

11. The test kit of claim **10**, wherein said channel is separable from said collection region prior to supplying the diluent to said collection region.

12. The test kit of claim **10**, wherein said channel is essentially non-conductive to the test sample.

13. The test kit of claim **10**, wherein said membrane is laminated to a support, said support defining a bottom surface of said channel.

14. The test kit of claim **10**, wherein said channel is treated with a hydrophobic agent.

15. The test kit of claim **1**, wherein said collection region comprises of saturation volume for the test sample that is less than about 25 microliters.

16. The test kit of claim **1**, wherein said collection region comprises of saturation volume for the test sample that is less than about 10 microliters.

17. A diagnostic test kit for detecting the presence of an analyte within a test sample, the test kit comprising a lateral flow assay device that further includes:

a membrane comprising a sample collection region and a detection region, said collection region physically defined so as to have a known saturation volume with respect to said test sample of less than about 100 microliters, such that upon saturation of said collection region, a known volume of the test sample is contained in said collection region;

a barrier disposed between said collection region and said detection region, wherein said barrier is removed to facilitate flow of said test sample from said collection region to said detection region of said membrane; and

a channel defined in said membrane to convey the test sample to said collection region, and further comprising means for seperating said collection region from said channel into its known saturation volume prior to supplying a diluent to said collection region.

18. The test kit of claim **17**, wherein said barrier comprises a soluble composition in a defined band across the width of said membrane, said soluble composition present

throughout said membrane in an amount to prevent migration of the test sample from said collection region to said detection region of said membrane.

19. The test kit of claim **18**, wherein said soluble composition is dissolvable by the diluent.

20. The test kit of claim **17**, wherein said barrier comprises a physical separation across said membrane, and further comprising a bridge configured to be placed across said divide so that the test sample flows from said collection region to said detection region with the diluent.

21. The test kit of claim **20**, wherein the test sample is whole blood, said bridge configured as a blood separation filter.

22. The test kit of claim **17**, further comprising a sampling tip disposed at an end of said channel, said tip configured for placement adjacent to the skin of a user for receipt of a test sample of blood.

23. The test kit of claim **17**, wherein said channel is essentially non-conductive to the test sample.

24. The test kit of claim **17**, wherein said channel is treated with a hydrophobic agent.

25. The test kit of claim **17**, wherein said collection region comprises of saturation volume for the test sample that is less than about 25 microliters.

26. The test kit of claim **17**, wherein said collection region comprises of saturation volume for the test sample that is less than about 10 microliters.

* * * * *

专利名称(译)	用于侧向流动测定装置的计量技术		
公开(公告)号	US7279136	公开(公告)日	2007-10-09
申请号	US11/301631	申请日	2005-12-13
[标]申请(专利权)人(译)	金伯利-克拉克环球有限公司		
申请(专利权)人(译)	金佰利WORLDWIDE，INC.		
当前申请(专利权)人(译)	金佰利WORLDWIDE，INC.		
[标]发明人	TAKEUCHI JAMES M SONG XUEDONG YANG KAIYUAN WEI NING FEASTER SHAWN R		
发明人	TAKEUCHI, JAMES M. SONG, XUEDONG YANG, KAIYUAN WEI, NING FEASTER, SHAWN R.		
IPC分类号	G01N21/00 B01L3/00 B32B5/02 C12M1/34 C12M1/36 C12M3/00 C12Q1/00 G01N15/06 G01N31/00 G01N31/22 G01N33/53 G01N33/566		
CPC分类号	G01N33/558 Y10S436/825 Y10T436/25625 Y10T436/108331 Y10T436/107497 Y10T436/25375		
其他公开文献	US20070134811A1		
外部链接	Espacenet USPTO		

摘要(译)

提供了用于检测驻留在测试样品中的分析物的诊断方法和相关的测试试剂盒。使用具有收集区域和检测区域的样品膜，收集区域具有用于预期测试样品的已知饱和体积。在收集区域和检测区域之间限定屏障。收集区域用体积小于约100微升的测试样品饱和，使得已知体积的测试样品包含在收集区域中。从膜的收集区域和检测区域之间移除屏障，并将稀释剂供应到膜的收集区域，以促进测试样品从收集区域流到膜的检测区域。