

Fig. 1

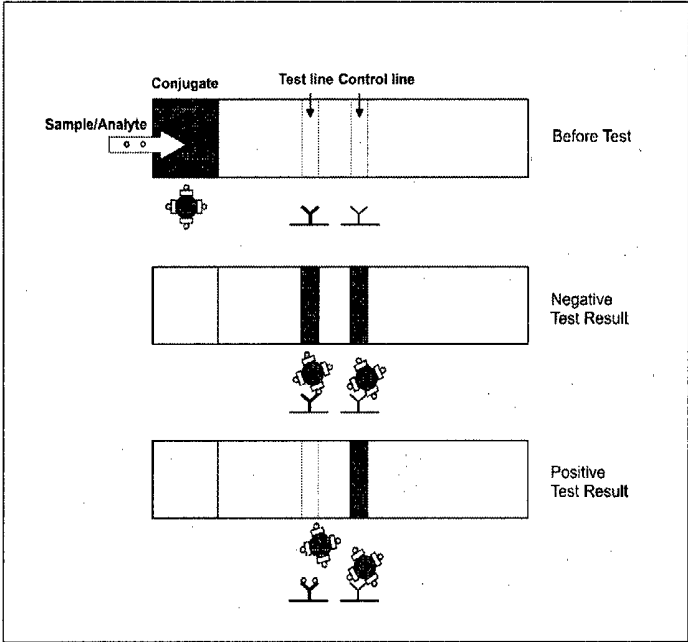


Fig.2

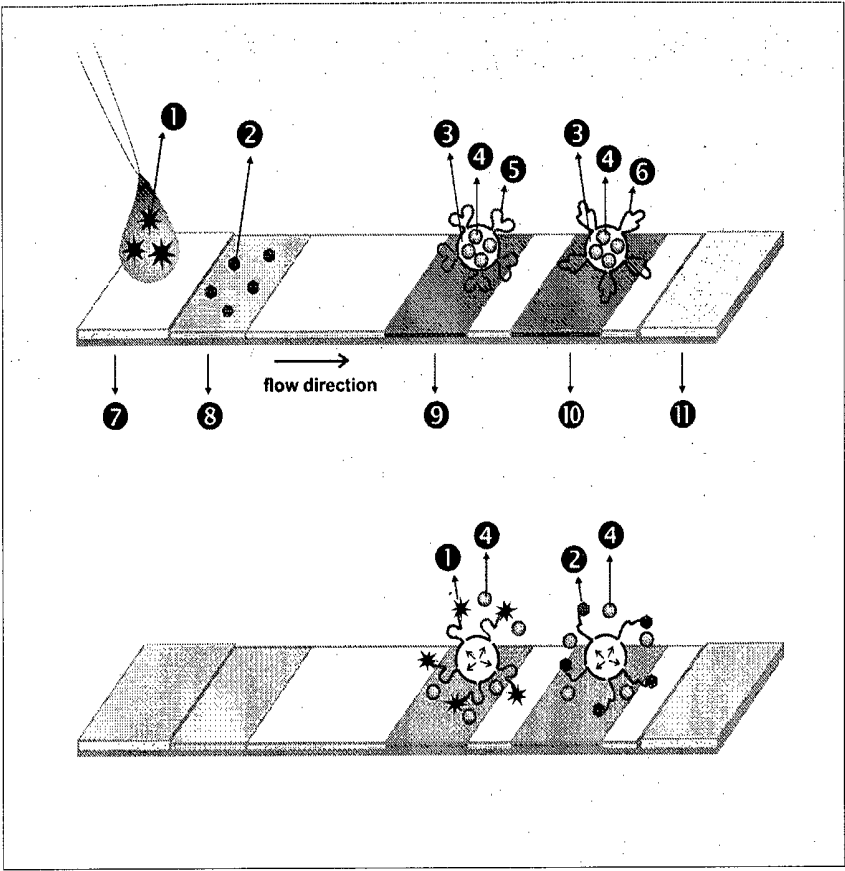


Fig.3

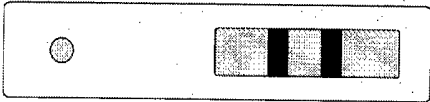
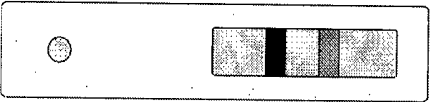

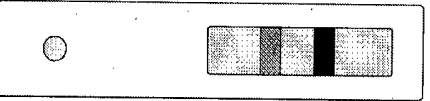

	Fluorescent Signal Molecule
Before the test	
Test result negative	
Test result positive	
Invalid test result	
	

Fig.4

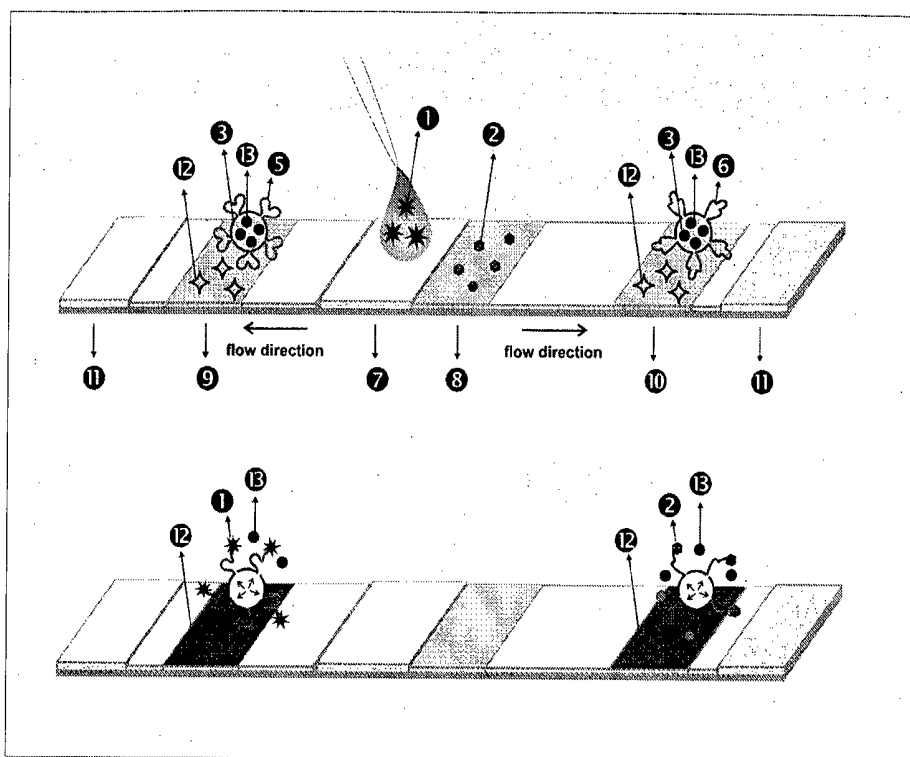


Fig.5


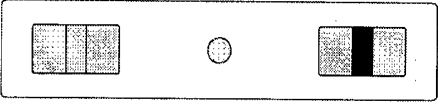

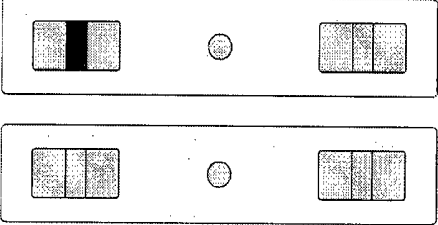
	Cromogenic Substrate Based Signal Molecule
Before the test	
Test result negative	
Test result positive	
Invalid test result	

Fig.6

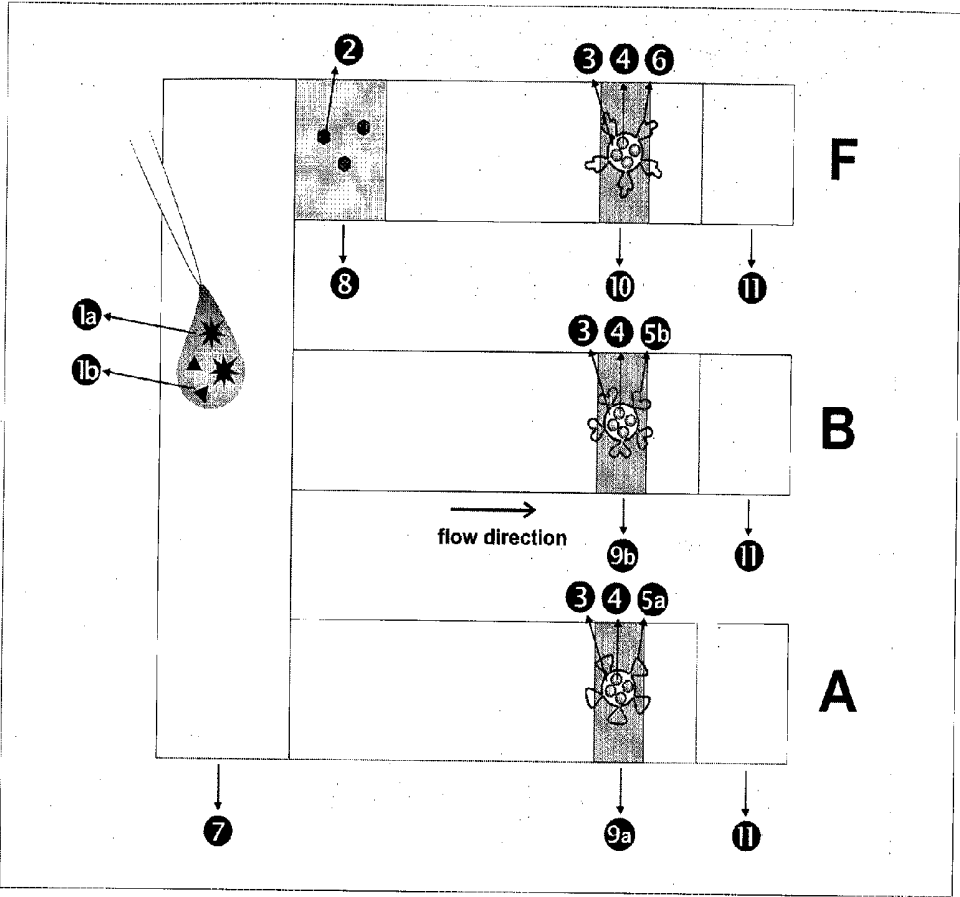


Fig.7A

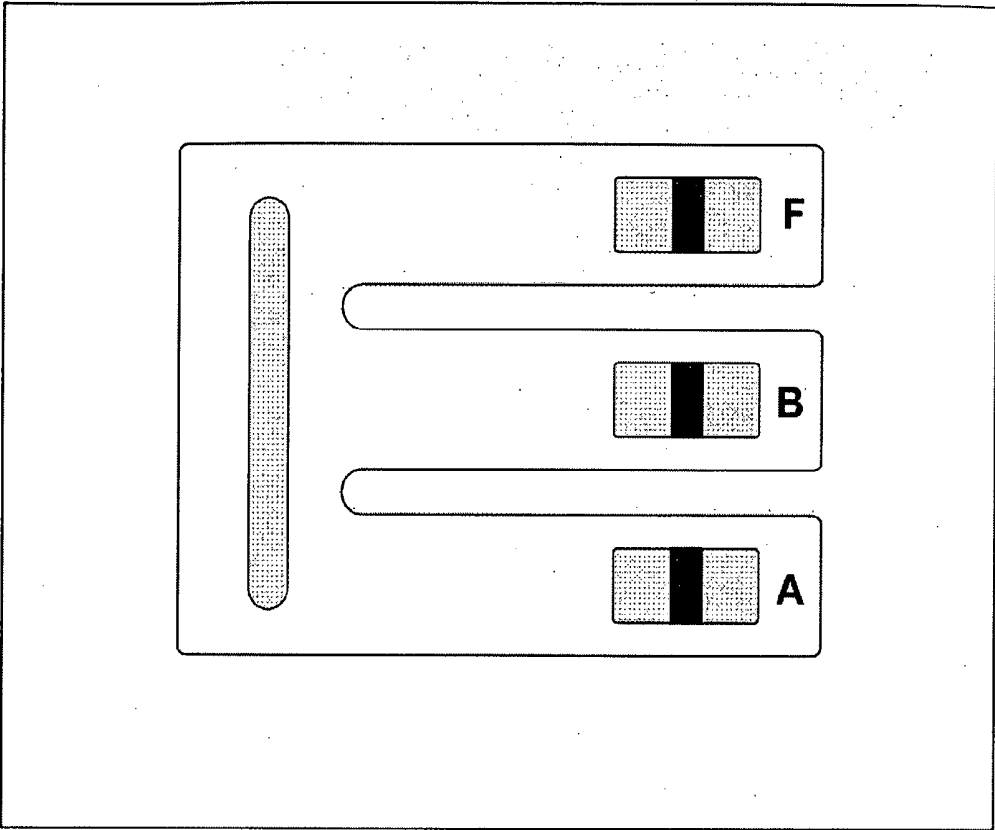


Fig.7B

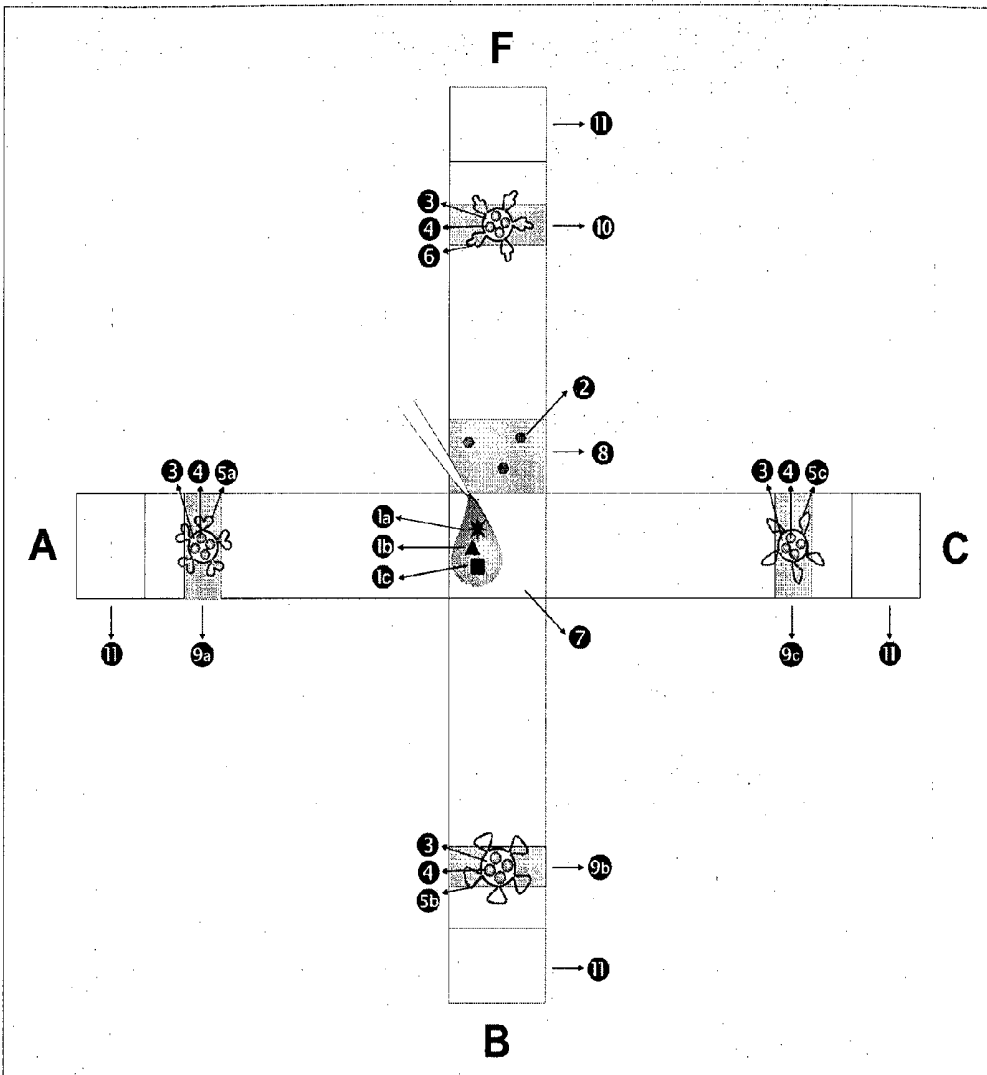


Fig.8A

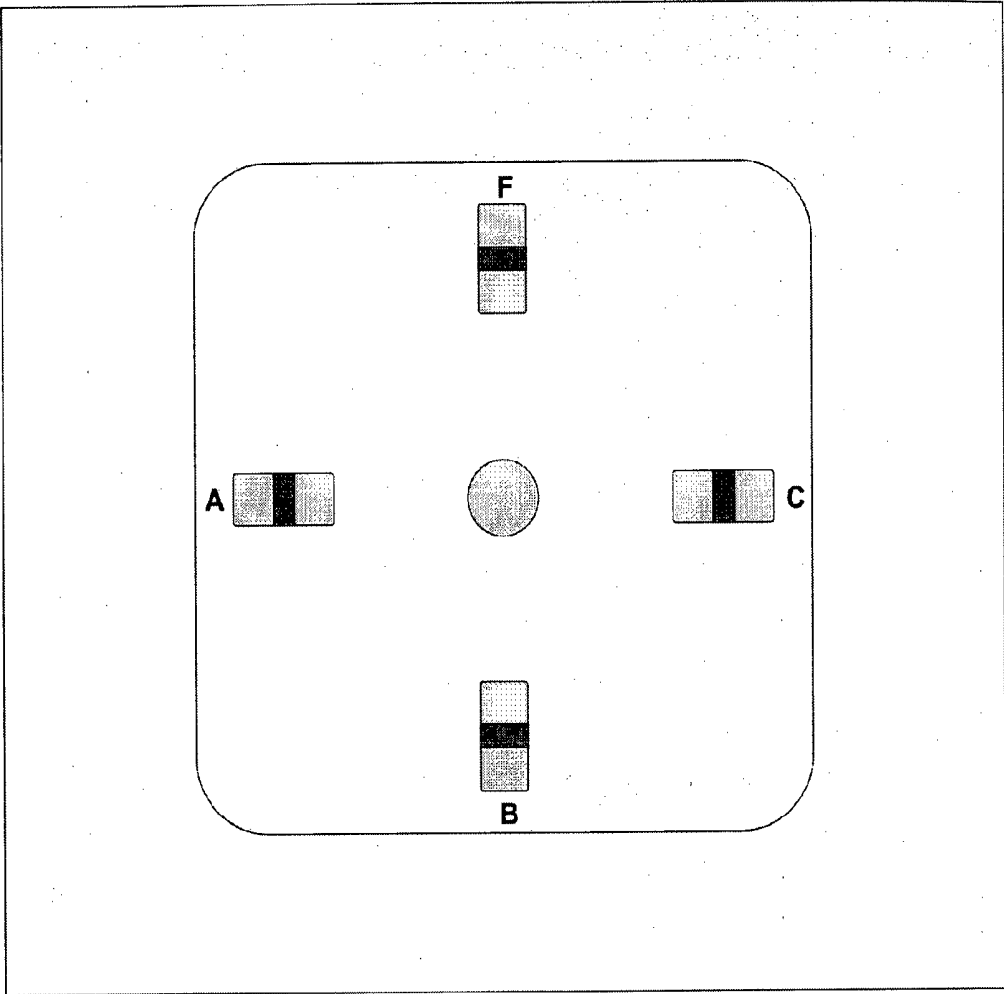


Fig. 8B

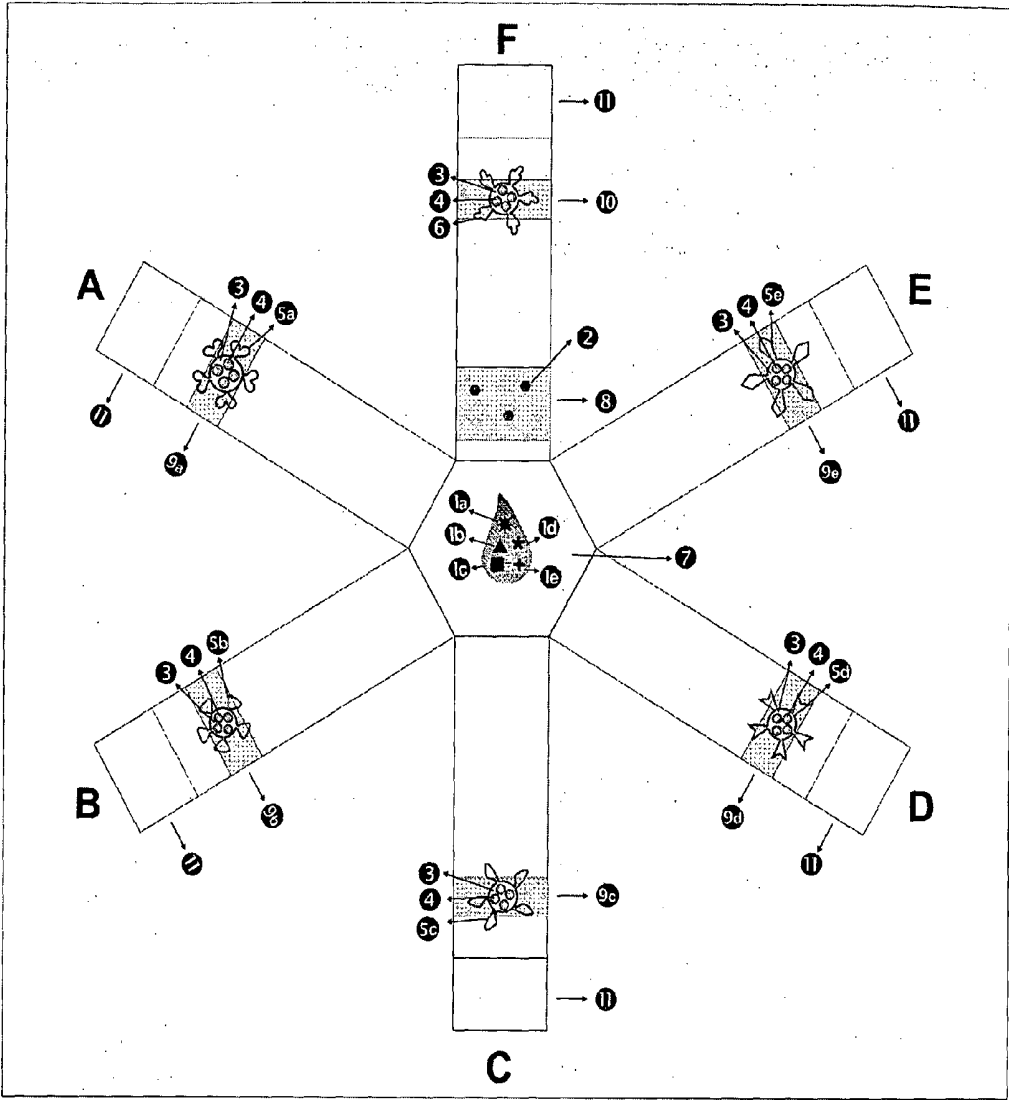


Fig. 9A

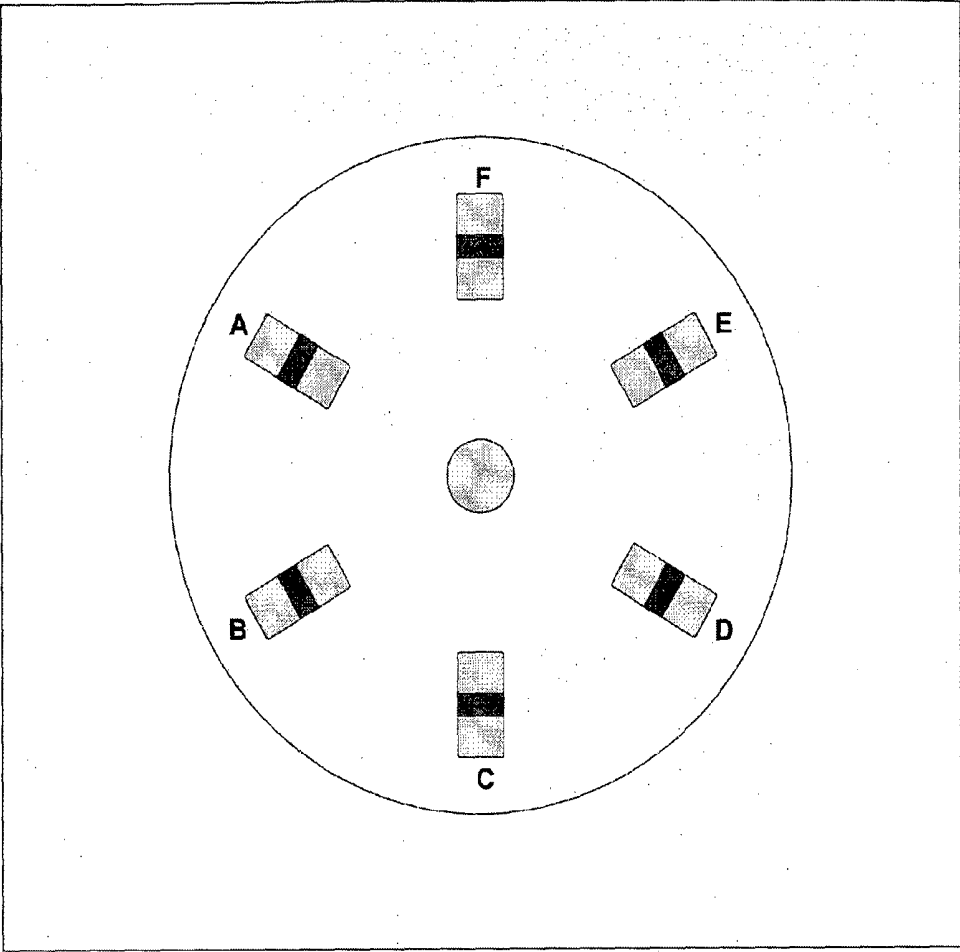


Fig.9B

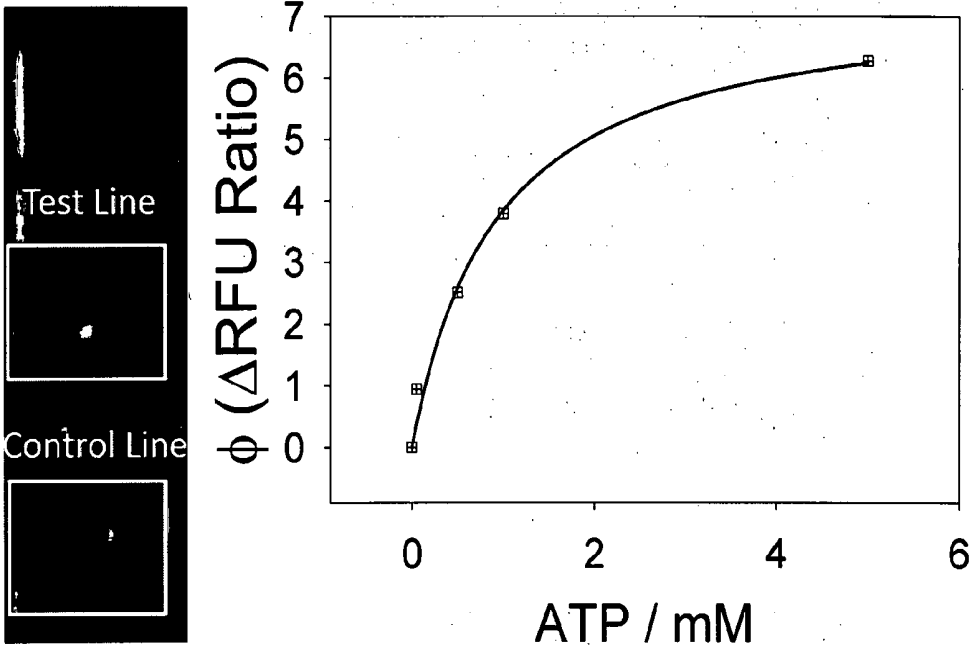


Fig.10

APTAMER-GATED NANOPARTICLES FOR LATERAL FLOW ASSAYS

TECHNICAL FIELD

[0001] The invention relates to a method for performing single step assays for the determination of the presence or absence of an analyte in a liquid sample, on a solid surface. The method disclosed, comprise an aptamer coated and signal molecule loaded porous silica particles immobilized on a porous solid material.

[0002] Specific interaction of the analyte with the aptamers coated on the silica beads, cause release of the signal molecules which result in a detectable signal on the solid support. Also provided are assay devices for the detection platform.

BACKGROUND OF THE INVENTION

[0003] Lateral flow (LF) immunoassay tests, also known as immunochromatographic strip tests, have been a popular platform for rapid immunoassays since their introduction in the mid-1980s.

[0004] The LF format is so versatile that manufacturers of rapid immunoassay tests have developed LF tests for almost any situation where a rapid test is required. In hospitals, clinics, physician offices, and clinical laboratories, LF-based tests are used for the qualitative and quantitative detection of the presence of a specific analyte in a liquid sample.

[0005] These test formats generally comprise a lateral flow test strip, such as nitrocellulose or a filter paper, a sample application area, test results area and an analyte specific binding reagent that is bound to some kind of detectable label, such as a colored particle, gold nanoparticle or an enzyme detection system.

[0006] The assay consists of several zones, typically constituted by segments made of different materials. When a test is run, sample is added to the proximal end of the strip, the sample pad. The sample migrates through this region to the conjugate pad, where a particulate conjugate has been immobilized. The conjugate comprise of a antibody or aptamer molecule immobilized to a particle. The particle can typically be colloidal gold, or a colored, fluorescent, or paramagnetic monodisperse latex particle.

[0007] This particle has been conjugated to one of the specific biological components of the assay, either antigen or antibody depending on the assay format. There are two common formats in LFA: direct and competitive.

[0008] The sample re-mobilizes the dried conjugate, and the analyte in the sample interacts with the conjugate as both migrate into the next section of the strip, which is the reaction matrix. This reaction matrix is a porous membrane, onto which the other specific biological component of the assay has been immobilized. These are typically proteins, either a bio-receptor (antibody or aptamer) or antigen, which have been laid down in bands in specific areas of the membrane where they serve to capture the analyte and the conjugate as they migrate by the capture lines. Excess reagents move past the capture lines and are entrapped in the wick or absorbent pad.

[0009] Results are interpreted on the reaction matrix as the presence or absence of lines of captured conjugate, read either by eye or using a reader.

[0010] The assay formats can be either direct (sandwich) or competitive (inhibition) and should be able to accommo-

date qualitative or semi-quantitative determinations. Direct assays are typically used when testing for larger analytes with multiple antigenic sites. Competitive formats are typically used when testing for small molecules with single antigenic determinants, which cannot bind to two antibodies simultaneously. In this format, a positive result is indicated by the absence of a test line on the reaction matrix. A control line should still form, irrespective of the result on the test line. Representative figures for direct or competitive assays are given in FIG. 1 and FIG. 2.

[0011] A large number of examples of such assay formats are available as literature data and patent applications. Some examples are as follows:

[0012] LFA for detection of prostate cancer markers has been defined (Cheng Fanga, Zhencheng Chenb, Lin Lib, Jinhong Xiab. Barcode lateral flow immunochromatographic strip for prostate acid phosphatase determination. *Journal of Pharmaceutical and Biomedical Analysis* 2011, 56, 1035-1040). LFA assays are also defined for detection of avian influenza virus (Xuepu Li, Donglian Lu, Zonghai Sheng, Kun Chen, Xuebo Guo, Meilin Jin, Heyou Han, A fast and sensitive immunoassay of avian influenza virus based on label-free quantum dot probe and lateral flow test strip. *Talanta* 2012, 100, 1-6). More comprehensive information can be also found in the following references (Rosen S., *Market Trends in Lateral Flow Immunoassays*, 35-50, (2009) *Lateral Flow Immunoassay*, Wong R. C., Harley Y., Tse H. Y., Eds., Humana Press), Geertruida A. Posthumatrumptie, Jakob Korf and Aart van Amerongen. *Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey Analytical and Bioanalytical Chemistry*, 2009, 393:569-582)

[0013] Although not as common as antibody based LFA formats, LFA's based on aptamer capture molecules has been also published. It is possible to detect proteins such as thrombin (Hui Xu, Xun Mao, Qingxiang Zeng, Shengfu Wang, Abdel-Nasser Kawde, and Guodong Liu. *Aptamer-Functionalized Gold Nanoparticles as Probes in a Dry-Reagent Strip Biosensor for Protein Analysis. Anal. Chem.* 2009, 81, 669-675), glycosylated albumin (Henry John Smith. *Aptamer based point-of-care test for glycosylated albumin*, US Patent application No: 20090042237, 2009) and cancer cells (Guodong Liu, Xun Mao, Joseph A. Phillips, Hui Xu, Weihong Tan, and Lingwen Zeng. *Aptamer-Nanoparticle Strip Biosensor for Sensitive Detection of Cancer Cells. Anal. Chem.* 2009, 81, 10013-10018)

[0014] Although LFA is a well-established test platform, it encounters various problems. One major problem is the sensitivity (limit of detection-LOD) for a given sample. Although strategies for signal amplification is possible thus lowering limit of detection is possible, these techniques are not practical and cost effective.

[0015] The other problem is the difficulties in developing assays with small molecules. In such assays due to the small nature and limited number of antigenic sites of the analyte it is not possible to establish direct (sandwich) assay formats. In case of competitive assays it is necessary to label or immobilize the small analyte which cause a reduction in the affinity of the assay (FIG. 2)

SUMMARY OF THE INVENTION

[0016] The present invention describes a new method of analyte recognition by using membrane immobilized, aptamer coated and signal molecule loaded mesoporous

silica beads. The method is applicable to both stationary dot blot assays and lateral flow assay platforms.

[0017] It is possible to identify analytes, including but not limited to small molecules (ATP, drugs, hormones, explosives etc.) nucleic acids, carbohydrates proteins, spores, prokaryotic cells (bacterial cells), eukaryotic cells (yeast, tumor cells etc.), and virus particles. The analytes can be detected in a variety of fluid samples including, but not limited to blood, tears, saliva, solid or liquid environmental samples.

[0018] The basic principle of the method is the interaction of the analyte (1) with specific aptamer molecules (2) immobilized on the silica beads (3) loaded with signal molecules (4). The specific interaction between the analyte and the aptamer causes opening of the pores and release of the signal molecules out of the silica beads, which generate a detectable signal (FIG. 3). Depending on the type of the signal molecule, the signal can be directly detected (as color, fluorescent etc.) or can be detected after processed by an enzyme (signal like TMB-3,3',5,5'-tetramethylbenzidine).

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1 Direct lateral flow assay format. Preferred method for analytes with multiple epitopes.

[0020] FIG. 2 Competitive lateral flow assay format. Preferred method for small analytes.

[0021] FIG. 3: Lateral flow assay test format utilizing aptamer coated and fluorescence signal molecule loaded silica particles.

[0022] 1—Target analyte

[0023] 2—Molecule to form a signal at control zone (10)

[0024] 3—Silica bead immobilized to the test and control zone (9)

[0025] 4—Signal molecule

[0026] 5—Specific aptamer for the target analyte (1)

[0027] 6—Specific aptamer to 2

[0028] 7—Sample application zone

[0029] 8—Control molecule zone

[0030] 9—Test zone

[0031] 10—Control zone

[0032] 11—Sample absorption pad

[0033] FIG. 4: Top view of the test device and interpretation of the test results when a fluorescence signal molecule is loaded to the silica particles.

[0034] FIG. 5: Lateral flow assay test format utilizing aptamer coated and chromogenic signal molecule loaded silica particles

[0035] 1—Target analyte

[0036] 2—Molecule to form a signal at control zone (10)

[0037] 3—Silica bead immobilized to the test (9) and control zone (10)

[0038] 5—Specific aptamer for the target analyte (1)

[0039] 6—Specific aptamer to (2)

[0040] 7—Sample application zone

[0041] 8—Control molecule (2) zone

[0042] 9—Test zone

[0043] 10—Control zone

[0044] 11—Sample absorption pad

[0045] 12—Molecule to process the signal molecule (13)

[0046] 13—Signal molecule (chromogenic substrate)

[0047] FIG. 6: Top view of the test device And interpretation of the test results when a chromogenic signal molecule is loaded to the silica particles.

[0048] FIG. 7A: Multiple test format device with 3 channels.

[0049] FIG. 7B: Top view of a test device with 3 channels.

[0050] FIG. 8A: Multiple test format device with 4 channels

[0051] FIG. 8B: Top view of multiple test format device with 4 channels

[0052] FIG. 9A: Multiple test format device with 6 channels

[0053] FIG. 9B: Top view of multiple test format device with 6 channels

[0054] FIG. 10: ATP titration results as measured with strips on the left. This is an example of LFA strip showing test line and control line before applying sample. The picture shows a fluorescent scan by exciting at 480 nm and reading emission at 520 nm (on the left). The results were plotted as ratio of fluorescent values of test line to control line after applying samples with different ATP concentrations. The control line consists of nanoparticles capped with a control aptamer with the same length and the same hairpin shape like ATP binding aptamer, but mutated in four nucleotides (underlined) to obtain a sequence that is not responsive to ATP.

DETAILED DESCRIPTION OF INVENTION

[0055] The invention provides a new assay platform that comprise an aptamer coated and signal molecule loaded silica particle, immobilized on a solid platform, a porous membrane, paper or fabric.

[0056] A “signal molecule” refers to a substance or compound that can be detected, particularly by visual, fluorescent or instrumental means. A signal molecule may be, for example, but not limited to, fluorescent molecule such as fluorescein or a pigment produced as a coloring agent or ink, such as Brilliant Blue, 3132 Fast Red 2R and 4230 Malachite Blue Lake.

[0057] The “signal molecule” may also be an enzyme substrate, such as, but not limited to, TMB or Bromo-Chloro-Indolylphosphate (BCIP) and Nitro Blue Tetrazolium (NBT/Thiazolyl Blue/Nitro BT). TMB (3,3',5,5'-tetramethylbenzidine) is a chromogenic substrate that yields a blue color when oxidized, typically as a result of oxygen radicals produced by the hydrolysis of hydrogen peroxide by horse radish peroxidase (HRP). For kinetic or non-stopped ELISA assays, the TMB chromogen has maximal absorbances at 370 nm and 652 nm. TMB is very sensitive ELISA substrate and is more quickly oxidized than other HRP substrates, resulting in faster color development.

[0058] The silica bead is a porous nanoparticle that can be chemically synthesized or commercially available from various chemical companies such as Sigma (Sigma, 643645, Silica, mesostructured, MCM-41 type (hexagonal) unit cell size: 4.5-4.8 nm pore size: 0.98 cm³/g pore volume, 2.1-2.7 nm pore size).

[0059] The aptamer molecule is nucleic acid sequence (DNA or RNA) that exhibits high affinity binding properties and specificity to a given analyte.

[0060] The analyte can be any target molecule, cell or virus particle present in a target sample. The analyte can be small molecules such as but not limited to nucleotides (ATP, GTP etc), drugs, pesticides, antibiotics, toxins, explosives,

hormones; biomolecules such as short peptides, proteins, carbohydrates, lipids and nucleic acids; bacterial cells, spores, fungal cells; eukaryotic cells such as yeast cells, tumor cells; virus particles and pollen particles.

[0061] The sample refers to a fluid such as, but not limited to blood, serum, plasma, saliva, tears, urine, semen, aqueous samples taken from environmental samples such as lakes, rivers, sea, water tanks, municipal, or industrial water sources, runoff water or sewage samples, food samples such as milk, yogurt; hard and soft drinks like wine, fruit juices and vegetable juices. Swap sample from any surface, throat or genital regions can be suspended in liquid solutions and can be also used as a sample.

[0062] Sample application zone part is the portion where a liquid sample including the analyte is applied. Depending on the type of the assay format various types of materials (filter paper, glass filter) can be used at this region of the test device to trap undesired molecules where the analyte moves towards the test zone. For instance when a blood sample is applied the blood cells can be hold by this zone. Alternatively molecules like, but not limited to charcoal (to trap phenolic compounds), silica particles (to trap nucleic acids), zeolite particles, aluminum silicate particles; can be included at this region to trap and prevent the interference of undesired molecules at the test zone.

[0063] Test zone part is the portion where the analyte interacts with the aptamer coated silica particles and induces the signal formation. Depending on the signal molecule the result can be visualized by naked eye or by the aid of an instrument.

[0064] Positive control zone is a test zone to verify the proper functioning of the assay. This zone includes silica particles coated with a particular aptamer, which is activated by a molecule that is by default present at the control molecule zone.

[0065] Although not shown in the figures, to estimate the non-specific release of the signal molecule, a negative test zone can be also incorporated to the assay. In such a format, a signal molecule loaded silica particle coated with a non-specific aptamer would be immobilized between test and control zones.

[0066] Working principle of the invention is as follows:

[0067] The present invention can be used to identify a variety of analytes in a given fluid sample. As shown in FIG. 3, the sample (in fluid form) containing the analyte (1) is applied to the sample application zone (7). The sample will move towards the test zone (9) and the sample absorption pad (11) by the capillary action of the membrane support. When the analyte reaches to the test zone (9) it interacts with the specific aptamers (5) coated onto the porous silica beads (3), which are immobilized to the test zone (9). The interaction of the analyte with the aptamer causes the opening of the pores of the silica particles, which in turn causes the release of the signal molecules (4) out of the silica particle thus generating a signal at the test zone.

[0068] During the capillary movement of the sample, a control molecule (2) present at the control molecule zone (8) also moves toward the absorption pad (11). When the control molecule reaches to the positive control zone (10) it interacts with its specific aptamer (6) coated on the porous silica particles (3) loaded with the signal molecule (4). This interaction also generates a signal, which indicates the

proper functioning of the assay. Alternatives for control molecule includes but not limited to a well defined analyte-aptamer pairs like:

[0069] ATP—CACCTGGGGGAGTATTGCGGAG-GAAGGTT (Huizenga, *Biochemistry*. 1995 17; 34(2):656-65)

[0070] Argininamide—CGACCAACGTGTCGCCTG-GTTCG (Harada K, Frankel A D (1995) *EMBO J* 14: 5798-5811)

[0071] Thrombin—GGTTGGTGTGGTTGG (Bock L C, Griffin L C, Latham J A, Vermaas E H, Toole J J. *Nature* 1992; 355:564-566.)

[0072] Alternatively, complementary nucleic acid sequences where one of the complementary sequence is coated on the silica bead and the other single strand complementary sequence is applied to the control molecule zone (8).

[0073] Depending on the nature of the signal molecule, type of the signal differs. In case of a florescent signal molecule, presence of analyte in the test sample causes a reduction in the fluorescent intensity due to the release of entrapped signal molecules at the test zone. FIG. 4 shows different possibilities and interpretation of the assay formats when a fluorescent signal is loaded into the silica beads.

[0074] The test formats using fluorescence signal molecules, needs instrumentation to be evaluated and it is not possible to evaluate the test results by visual inspection.

[0075] The present invention can be also used to obtain test results, which can be evaluated without the use of any instrument. In such test formats (FIG. 5) the results can be evaluated by visual inspection by naked eyes.

[0076] The assay format comprise a single strip where the sample to be evaluated is applied to the sample application pad (8) which is located at the center of the strip. The sample with the analyte (1) moves towards sample absorption pads (11) present at both ends of the strip by the capillary action. When the analyte reaches to the test zone (10) it interacts with the specific aptamers (5) coated onto the porous silica beads (3) immobilized at the test zone (9) The interaction of the analyte with the specific aptamer (5) causes the opening of the pores of the silica particles, which in turn causes the release of the signal molecule (13). The signal molecule (13) in this case can be a chromogenic molecule or a chromogenic substrate for an enzyme (12), which is also immobilized at the test zone (10) or close vicinity of the test zone.

[0077] In case of a chromogenic signal molecule a visual signal directly appears at the test zone after the release of the chromogenic molecule from the silica beads. This signal can be detected by visual inspection of the test zone (FIG. 6)

[0078] If the signal molecule is a chromogenic substrate, after the release from the silica beads, the substrate is processed by the enzyme (12) present at the test zone to form a visual signal that can be detected by visual inspection of the test zone (FIG. 5).

[0079] In order to evaluate the proper functioning of the test, a positive control zone (10) is also established at the opposite side of the strips. During the capillary movement of the sample towards the absorption pad (12), a control molecule (2) present at the control molecule zone (8) also moves toward the absorption pad (12). When the control molecule reaches to the positive control zone (10) it interacts with its specific aptamer (6) coated on the porous silica particles (3) loaded with the signal molecule (13). This interaction also causes the release of the signal molecule out

of the porous silica particles (3) at the positive control zone (10) which, in turn forms a direct visual signal (in case the signal molecule is a chromogenic molecule) or after processes by the enzyme (12) located at the positive control zone (in case the signal molecule is a chromogenic substrate). This signal is an indicator of the proper functioning of the assay. Possible assay outcomes and their interpretation is shown in FIG. 6.

[0080] Present invention can be also used to detect various analytes at the same time (FIG. 7-9). In such test formats there is a single sample application zone (7), which is connected to multiple test channels (A-E) and a control channel (F). Each test channel contains a test zone (9a-9e). Depending on the number of different analytes to be detected the number of the test channels can be multiplied. Each test zone (9a-e) contains silica beads coated with different specific aptamers for a given analyte that are intended to be analyzed by the test device. Multiple test zone test formats also contain a control channel (F). At the entrance of the test channels there is the control molecule pad (8) with the, control molecule (2). The control channel is also equipped with signal molecule loaded silica beads coated with aptamers specific to the control molecule. These beads are immobilized to the control zone (10). All of the channels have a sample adsorption pad at their distal ends (11).

[0081] Present invention has versatile application areas including but not limited to health, food security, homeland security, bioterrorism, environmental monitoring, veterinary applications, beverage industry and others. Although it is not limited with those, some representative examples for the application of the present invention is given below.

[0082] Small Molecule Detection (ATP)

[0083] The present invention may be used to identify ATP molecules in a given sample. The silica particles with signal molecule inside and aptamers on the surface covering the pore openings were previously reported to detect a small molecule in solution form (Ozalp, V. C. & Schafer, T. Chem. Eur. J. 2011, 17, 9893-9896; Chun-Ling Zhu; Chun-Hua Lu; Xue-Yuan Song; Huang-Hao Yang; Xiao-Ru Wang; J. Am. Chem. Soc. 2011, 133, 1278-1281). Fluorescein molecules (signal molecule) was loaded in silica nanoparticle porous containers and covered with ATP responsive aptamer sequences as described in Ozalp and Schafer (Ozalp, V. C. & Schafer, T. Chem. Eur. J. 2011, 17, 9893-9896). ATP binding aptamer sequence was originally selected by Huizenga (D. E. Huizenga, J. W. Szostak, Biochemistry 1995, 34, 656-665). The aptamer sequence was converted into a molecular-beacon-type hair pin structure by adding additional nucleotides at one end of the sequence. The final hairpin structure was as follows:

(5' - *CACCTGGGGAGTATTGCCGGAGGAAGGTTCCAGGTG* -NH₂-3')
(italic sequence is the added nucleotides for converting the aptamer to hairpin)

[0084] Mesoporous silica particles (MCM-41) of hexagonal architecture with a unit size of 4.6 to 4.8 nm and an average pore size of 2.3 to 2.7 nm were employed for preparing ATP responsive release system. The aptamer hairpin structures were covalently conjugated to the silica surface of nanoparticles. The duplex neck region of the hairpin structure of the ATP-binding aptamer DNA when attached to the surface of nanoparticles, could successfully block the release of guest molecules in mesoporous silica particles. In

the present invention, fluorescein loaded silica nanoparticles capped with ATP recognizing aptamer sequences in hairpin structure were immobilized on test regions of LFA strips through a linker (Ozalp V. C., Analyst, 2013, 138, 4255) or by applying polyacrylamide embedding. FIG. 10 shows that when samples containing ATP were applied on the sample pad, ATP molecules flow through test and control lines. Since ATP causes opening of aptamer gates, fluorescein inside the nanopores are released and washed by flowing buffer. The release of fluorescein can be monitored by quantifying the decrease of fluorescein signal in the test line as described in detail elsewhere (Ozalp V. C., Analyst, 2013, 138, 4255). The absolute decrease of fluorescein was plotted against concentration of ATP samples to obtain a binding curve fitted to Langmuire type binding kinetics (FIG. 11).

5' - *CACCTAGGAGAGTAATGCCGAGGAAGGTTCCAGGTG* -NH₂-3'
(Underlined are mutated nucleotides)

[0085] Virus Particle Detection (HIV)

[0086] As an example to the application in the health sector, the invention can be used to detect HIV viruses in blood samples and body fluids. For such applications few drops of the sample (blood or saliva) is applied to the sample pad. The sample pad contains specific materials, which absorbs or filtrates the blood cells. To achieve the specific detection, the signal molecule loaded porous silica beads can be coated with HIV specific aptamers. For example, RNA aptamer recognizing TAT protein as shown below:

TAT a ptamer:
ACGAAAGCUUGAUCCCGUUUGCCGGUCGAUCGCUUCGA

[0087] Similarly, DNA aptamer sequences were selected to bind specifically to HIV reverse transcriptase enzyme.

5' - *AGCAGCACAGAGGTCAGATGGCAGGTTTCGACGTACAATGCTATGGAGGCTTTATGATCGCCTATGCGTGCTACCGTGAA* -3'

[0088] An example application to veterinary field is given below.

[0089] Bovine viral diarrhoea is a viral disease of cattle and other ruminants which reduces productivity and increases death loss. It is caused by the bovine viral diarrhoea virus (BVDV). BVDV is a member of the pestivirus genus. There are four recognized species within the pestivirus genus. These species are BVDV-1, BVDV-2, border disease virus of sheep and classical swine fever virus, previously known as hog cholera virus.

[0090] In the literature aptamers specific to bovine viral diarrhoea virus (BVDV) type 1 virus has been defied (Jee-Woong Park, Su Jin Lee, Eun-Jin Choi, Jaejo Kim, Jae-Young Song, Man Bock Gu Biosensors and Bioelectronics 51 (2014) 324-329). Two of such aptamer sequences are given below.

5' - Bio - *CGTACGGAATTCGCTAGCTGCGCATCCACAAATGTATTGTGGGGATGGATCCGAGCTCCACGTG* -3'

5' - Thiol - *CGTACGGAATTCGCTAGCTGCGCATCCACAAATGTATTGTGGGGATGGATCCGAGCTCCACGTG* -3'

[0091] The invention can be used for the detection of BVDV type 1 on a lateral flow assays. As described in

different parts of the document the mesostructured silica particles can be loaded with a signal molecule like 3,3',5,5'-Tetramethylbenzidine (TMB) and lateral flow strips can be prepared as shown in FIG. 3.

[0092] These silica particles with signal molecule inside and aptamers on the surface covering the pore openings will be applied on the lateral flow strips as shown in FIGS. 1 and 3. If the silica particles are loaded by TMB, the test line will contain HRP. Sample will be prepared with proper amount of H₂O₂ and applied to the sample pad. If the sample contains the virus particles, then the aptamer on silica particle surface will specifically interact with the aptamer and left the pores open. As a result there will be a release of TMB, and the blue color forming oxidation reaction will occur with the enzyme HRP and H₂O₂ inside the sample.

[0093] Eukaryotic Cell Detection (Tumor Cells)

[0094] Another application is identification of a target cell like a tumor cell. For such an application signal molecule loaded silica nanoparticles would be coated by an aptamer specific to nucleolin protein. The sequence of the nucleolin aptamer AS1411 was previously published (P. J. Bates, D. A. Laber, D. M. Miller, S. D. Thomas and J. O. Trent, Exp. Mol. Pathol., 2009, 86, 151-164). The AS1411 aptamer is a G-quadruplex forming a 26-base DNA sequence that has high affinity and specificity for nucleolin protein. The aptamer sequence is as follows:

5' -GGTGGTGGTGGTGGTGGTGGTGG-3'

[0095] Nucleolin is a multifunctional protein that can interact with both DNA and RNA molecules. It is present in the nucleolus and cytoplasm of most healthy cells and its expression is highly elevated on the surface of rapidly proliferating cancer cells. Therefore, in the case of the presence of a tumor cell in a particular sample, the cells interacts with the aptamer coated silica particles which causes the release of the signal molecule. This in turn causes the generation of a detectable signal on the lateral flow device.

[0096] Bacterial Pathogen Detection (*Salmonella* Detection)

[0097] An example for food security area is as follows. *Salmonellae* are a significant cause of food borne illness world-wide. Among a multitude of epidemic enteric bacterial infections, salmonellosis is one of the most frequently reported bacterial foodborne diseases and is a major economic and public health concern worldwide. Of particular concern is salmonellosis caused by multidrug-resistant strains such as *Salmonella enterica* serovar *Typhimurium* or *S. enterica* serovar Newport (Gupta, A.; Fontana, J.; Crowe, C.; Bolstorff, B.; Stout, A.; Van Duyne, S.; Hoekstra, M. P.; Whichard, J. M.; Barrett, T. J.; Angulo, F. J.: J. Infect. Dis. 2003, 188, 1707-1716). In addition, *S. typhimurium* is a major causative agent of gastroenteritis (characterized by diarrhea, cramps, vomiting, and often fever) in many areas (Jones, B. D.; Ghori, N.; Falkow, S.: J. Exp. Med. 1994, 180, 15-23). *Salmonellae* species are also important bacterial pathogens in food animal species including chickens, cows, turkeys and pigs. Amongst non-human cases, *S. typhimurium* is reported as the most causative agent of infections. The present invention can be also used to detect *S. typhimurium* presence in a given sample.

[0098] Silica particles were incubated overnight in phosphate buffered saline solution loaded with the signal mol-

ecule (TMB or FAM). Silanization of the surface will be followed by immobilization of the specific aptamer molecules as reported in the literature (Raghavendra Joshi, Harish Janagama, Hari P. Dwivedi, T. M. A. Senthil Kumar, Lee-Ann Jaykus, Jeremy Schefers, Srin and Sreevatsan. Molecular and Cellular Probes 23 (2009) 20-28.7). On of such aptamer molecule sequence is as follows:

5' -TAT GGC GGC GTC ACC CGA CGG GGA CTT-3'

[0099] These silica particles with signal molecule inside and aptamers on the surface covering the pore openings will be applied on the lateral flow strips as shown in FIGS. 1 and 3. If the silica particles are loaded by TMB, the test line will contain HRP. Sample will be prepared with proper amount of H₂O₂ and applied to the sample pad. If the sample contains the *Salmonella* species, then the aptamer on silica particle surface will specifically interact with the aptamer and left the pores open. As a result there will be a leakage of TMB, and the blue color forming oxidation reaction will occur with the HRP in close position and H₂O₂ inside the sample. On the other hand, in the absence of *Salmonella* in the sample solution, no pore opening will happen thus no signal formation occurs.

[0100] Protein Detection (Ricin)

[0101] The invention can be used to identify presence of target protein molecules in sample of interest. One such application would be detection of a bioterror agent ricin in a given sample. If the provided sample is not fluid, it might be suspended in phosphate buffered saline (PBS) solution and then applied to the test device. All of the steps of the procedures would be the same as explained previously in different parts of this document, except for the aptamers that is coated on the surface of the silica beads. The aptamer molecule would be a sequence that is already published in the literature (Elise A. Lamont, Lili He, Keith Warriner, Theodore P. Labuzab and Srinand Sreevatsan, Analyst, 2011, 136, 3884-3895). The sequence of the aptamer for ricin is as follows:

5' ACACCCACCGCAGGCAGACGCAACGCCTCGGAGACTAGCC 3'

1. Lateral Flow assay platform comprising application zone (7), at least one test zone (9) where aptamer (5) coated and signal molecule (4) loaded particle (3) is immobilized, control zone (8) where a control molecule (2) is immobilized and positive control zone (10)

2. Lateral Flow assay platform according to claim 1 where signal molecule (4) is fluorescent molecule.

3. Lateral Flow assay platform according to claim 2 where signal molecule (4) is fluorescein

4. Lateral Flow assay platform according to claim 1 where signal molecule (4) is chromogenic molecule

5. Lateral Flow assay platform according to claim 4 where signal molecule (4) is enzyme substrate

6. Lateral Flow assay platform according to claim 5 where signal molecule (4) is TMB (3,3',5,5'-tetramethylbenzidine)

7. Lateral Flow assay platform according to claim 4 where signal molecule (4) is Bromo-Chloro-Indolylphosphate (BCIP) or Nitro Blue Tetrazolium (NBT/Thiazolyl Blue/Nitro BT)

8. Lateral Flow assay platform according to claim 1 where particle (3) is porous silica bead.

9. Lateral Flow assay platform according to claim 1 where control molecule (2) is ATP, Argininamide, thrombin or a single stranded nucleic acid sequence complementary to nucleic acid sequence coated on the silica beads (3) present at the control zone (10)

10. A method for detecting an analyte by using Lateral Flow assay platform according to claim 1 comprises,

- a) Applying sample to application zone (7)
- b) Interaction of analyte (1) with specific aptamer (5) coated silica beads (3) on test zone (9)
- c) Opening the pores of silica particles (3)
- d) Release of the signal molecule (4)
- e) Generating a signal at the test zone (9)
- f) forwarding control molecule (2) on control zone (8) to positive control zone (10)
- g) Interaction of control molecule (2) with specific aptamer (6) coated silica beads (3) on positive control zone (10)

11. A method according to claim 10 where sample is blood, serum, plasma, saliva, tears, urine, semen.

12. A method according to claim 10 where sample is aqueous samples taken from lakes, rivers, sea, water tanks, municipal, or industrial water sources, runoff water or sewage samples,

13. A method according to claim 10 where sample is food samples from meat, vegetables, fruits, milk, yogurt; hard and soft drinks, fruit juices and vegetable juices.

14. A method according to claim 10 where sample from throat or genital regions.

15. A method according to claim 10 where analyte is small molecule

16. A method according to claim 15 where analyte is nucleotides (ATP, GTP etc), drugs, narcotic drugs, pesticides, antibiotics, toxins, explosives, hormones.

17. A method according to claim 10 where analyte is biomolecules

18. A method according to claim 17 where analyte is short peptides, proteins, carbohydrates, lipids and nucleic acids.

19. A method according to claim 10 where analyte is bacterial cells, spores fungal cells eukaryotic cells, tumor cells.

20. A method according to claim 10 where analyte is virus particles or pollen particles.

* * * * *

专利名称(译)	用于侧向流动测定的适体门控纳米颗粒		
公开(公告)号	US20160299136A1	公开(公告)日	2016-10-13
申请号	US15/103879	申请日	2013-12-13
[标]申请(专利权)人(译)	NANObiz NANOBIYOTEKNOLOJIK教育系统的IT咨询公司R D所TIC LTD.STI		
申请(专利权)人(译)	NANObiz NANOBIYOTEKNOLOJIK教育系统IT咨询研发SAN.TIC. LTD. STI.		
当前申请(专利权)人(译)	NANObiz NANOBIYOTEKNOLOJIK教育系统IT咨询研发SAN.TIC. LTD. STI.		
[标]发明人	OZALP VELI CENGIZ OKTEM HUSEYIN AVNI HERNANDEZ FRANK J HERNANDEZ LUIZA I SCHAFFER THOMAS		
发明人	OZALP, VELI CENGIZ OKTEM, HUSEYIN AVNI HERNANDEZ, FRANK J. HERNANDEZ, LUIZA I. SCHAFFER, THOMAS		
IPC分类号	G01N33/558 G01N33/552 G01N33/543 G01N33/53		
CPC分类号	G01N33/558 G01N33/54313 G01N33/552 G01N33/5308 C12N15/111 C12N15/115 C12N2310/16 C12N2320/10 C12Q1/6816		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及一种用于在固体表面上进行用于确定液体样品中分析物的存在或不存在的单步测定法的方法。所公开的方法包括固定多孔固体材料上的适体包被的和信号分子负载的多孔二氧化硅颗粒。分析物与涂覆在二氧化硅珠粒上的适体的特异性相互作用导致信号分子的释放，其在固体支持物上产生可检测的信号。还提供了用于检测平台的测定装置。

