US 20100047816A1

(19) **United States**

(12) **Patent Application Publication**

Badwan et al.

(10) **Pub. No.: US 2010/0047816 A1**

(43) **Pub. Date: Feb. 25, 2010**

(54) **RAPID IMMUNOCHROMATOGRAPHIC DETECTION BY AMPLIFICATION OF THE COLLOIDAL GOLD SIGNAL**

(75) Inventors: **Adnan Badwan**, Amman (JO); **Murshed Abdel-Qader Mohammed**, Amman (JO)

Correspondence Address:
PATENT CENTRAL LLC
Stephan A. Pendorf
1401 Hollywood Boulevard
Hollywood, FL 33020 (US)

(73) Assignees: **The Jordanian Pharmaceutical Manufacturing Co.**, Naor (JO); **Terramark Markencreation GmbH**, Bremen (DE)

(21) Appl. No.: **12/518,752**

(22) PCT Filed: **Dec. 6, 2007**

(86) PCT No.: **PCT/EP2007/010627**

§ 371 (c)(1),
(2), (4) Date: **Oct. 26, 2009**

(30) **Foreign Application Priority Data**

Dec. 11, 2006 (EP) 06025529.6

Publication Classification

(51) **Int. Cl.**
G01N 33/53 (2006.01)
C12M 1/34 (2006.01)
G01N 33/566 (2006.01)
G01N 30/00 (2006.01)

(52) **U.S. Cl.** **435/7.1**; 435/287.2; 436/501; 422/69

(57) **ABSTRACT**

The present invention relates to a rapid immunochromatographic test device suitable to detect an antibody and/or antigen in at least one sample, uses of said device for detecting diseases in a sample, a method for the production of said device as well as a kit comprising the device.

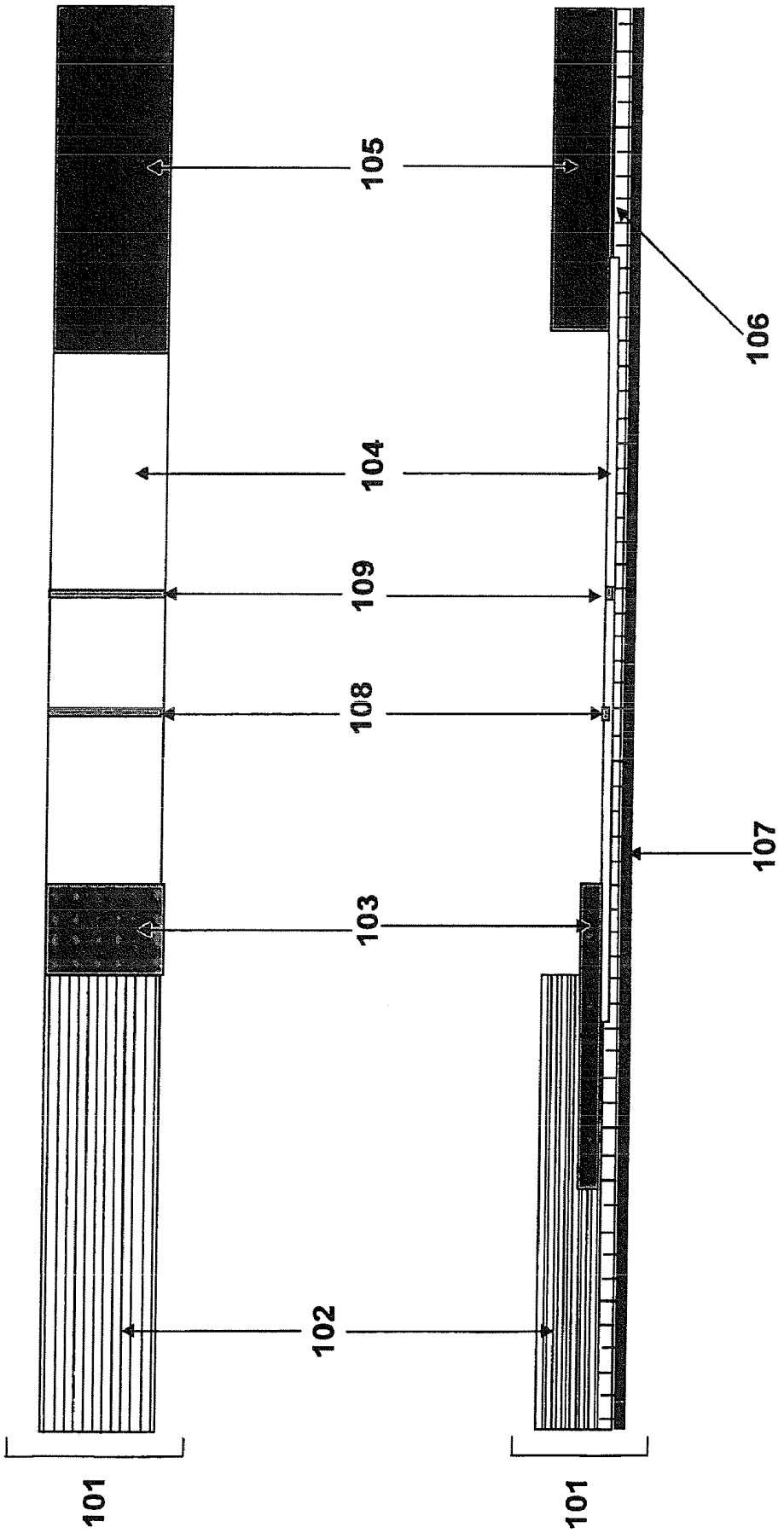


Figure 1a

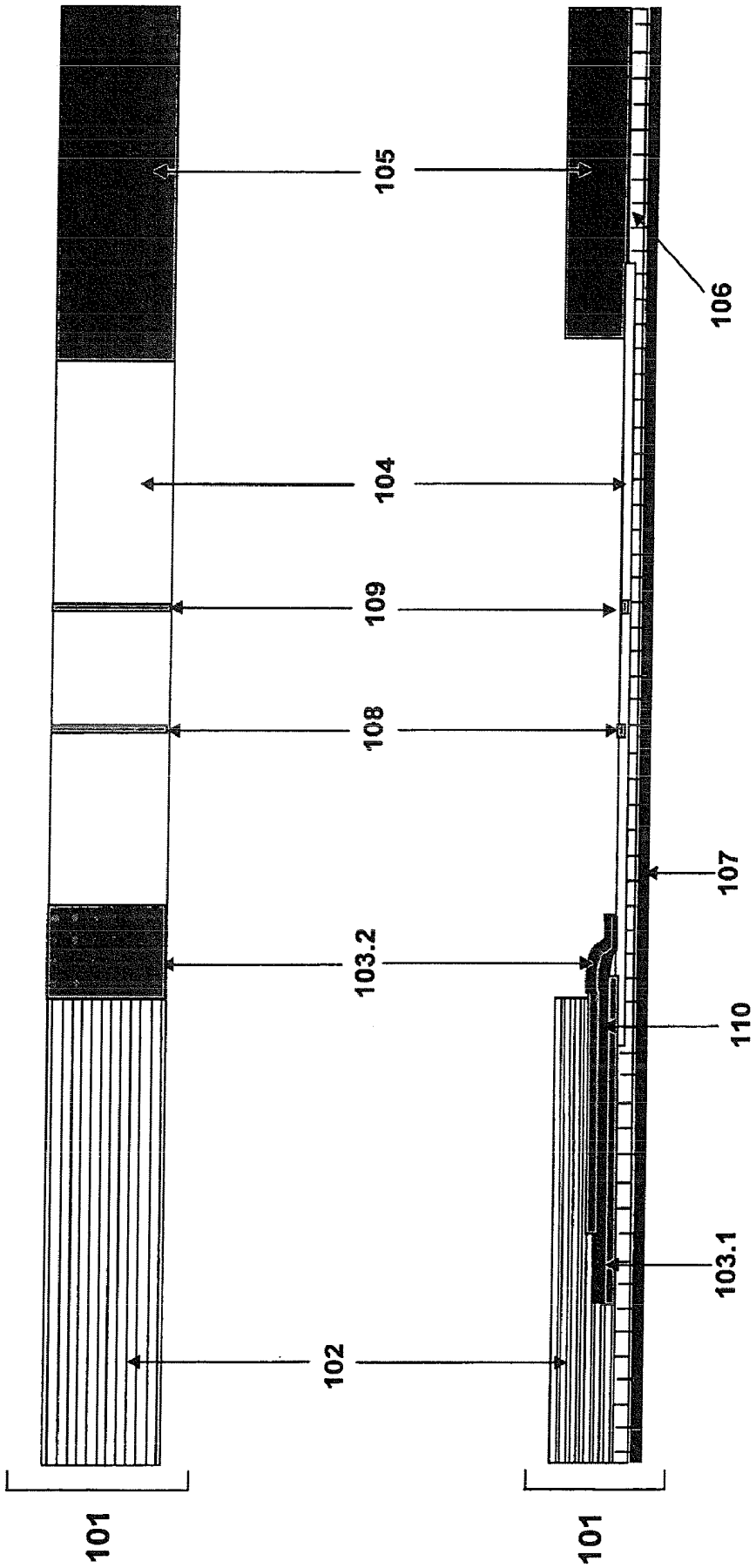


Figure 1b

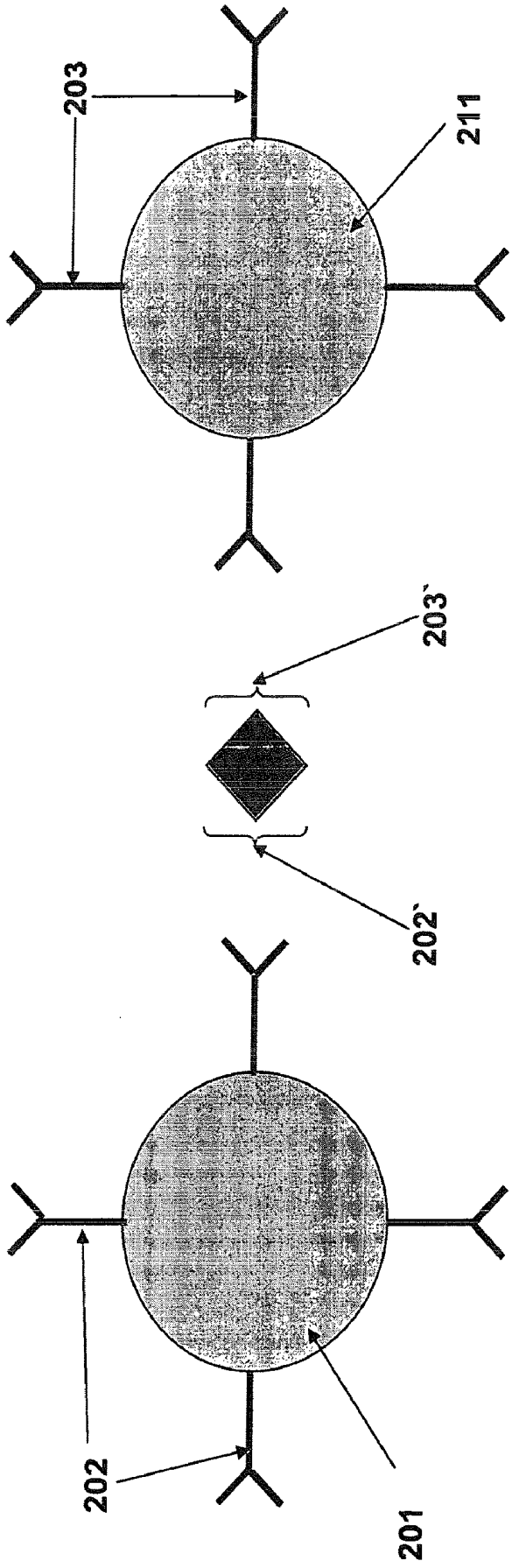


Figure 2

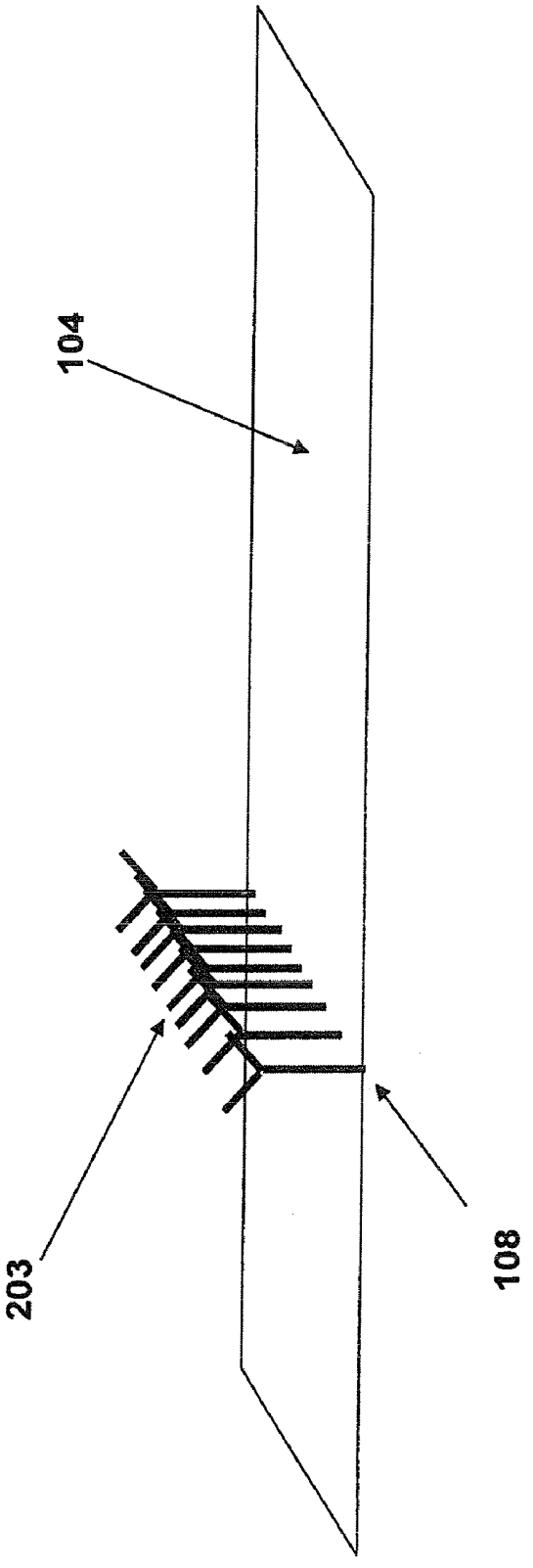
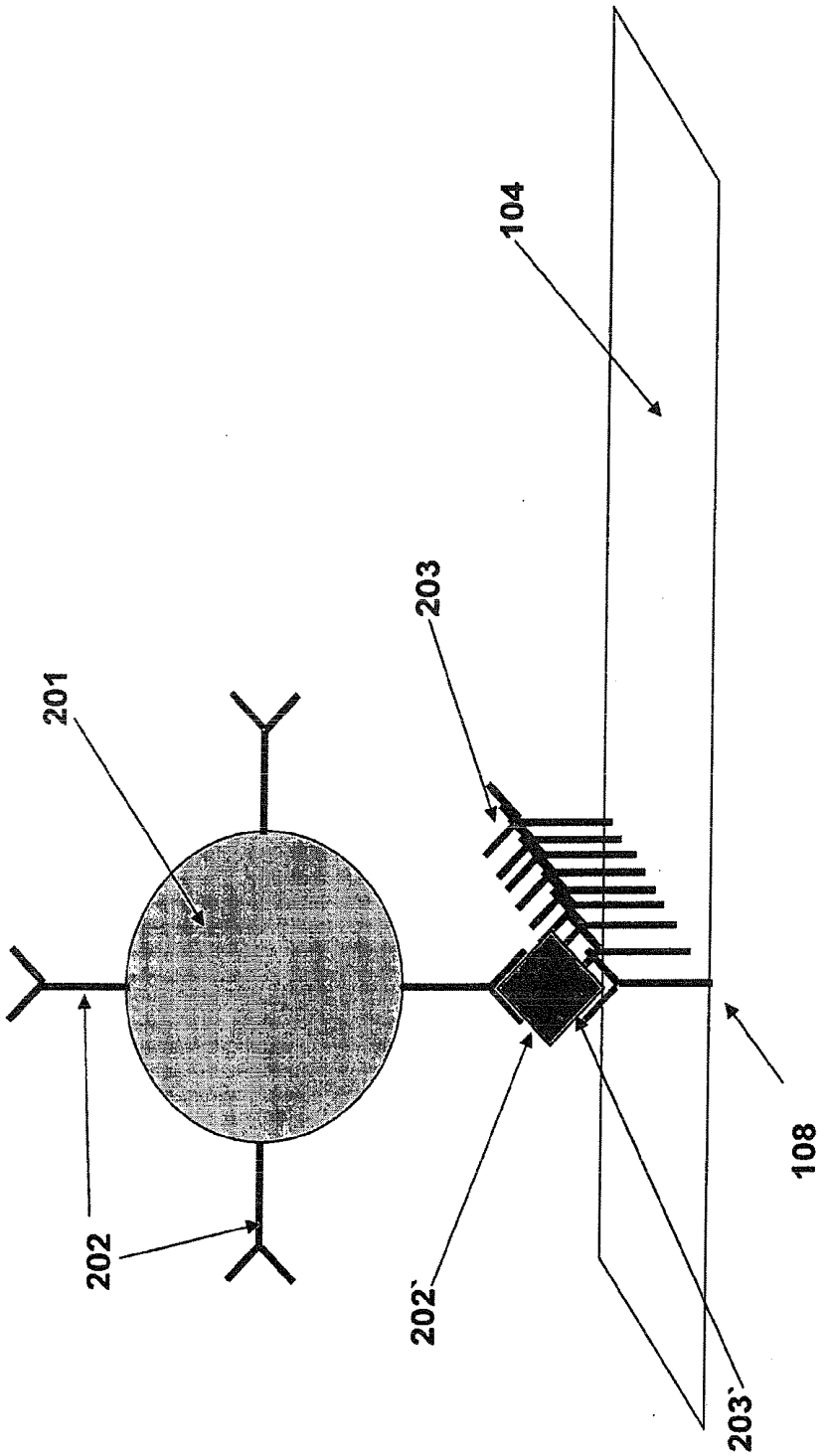


Figure 3

Figure 4



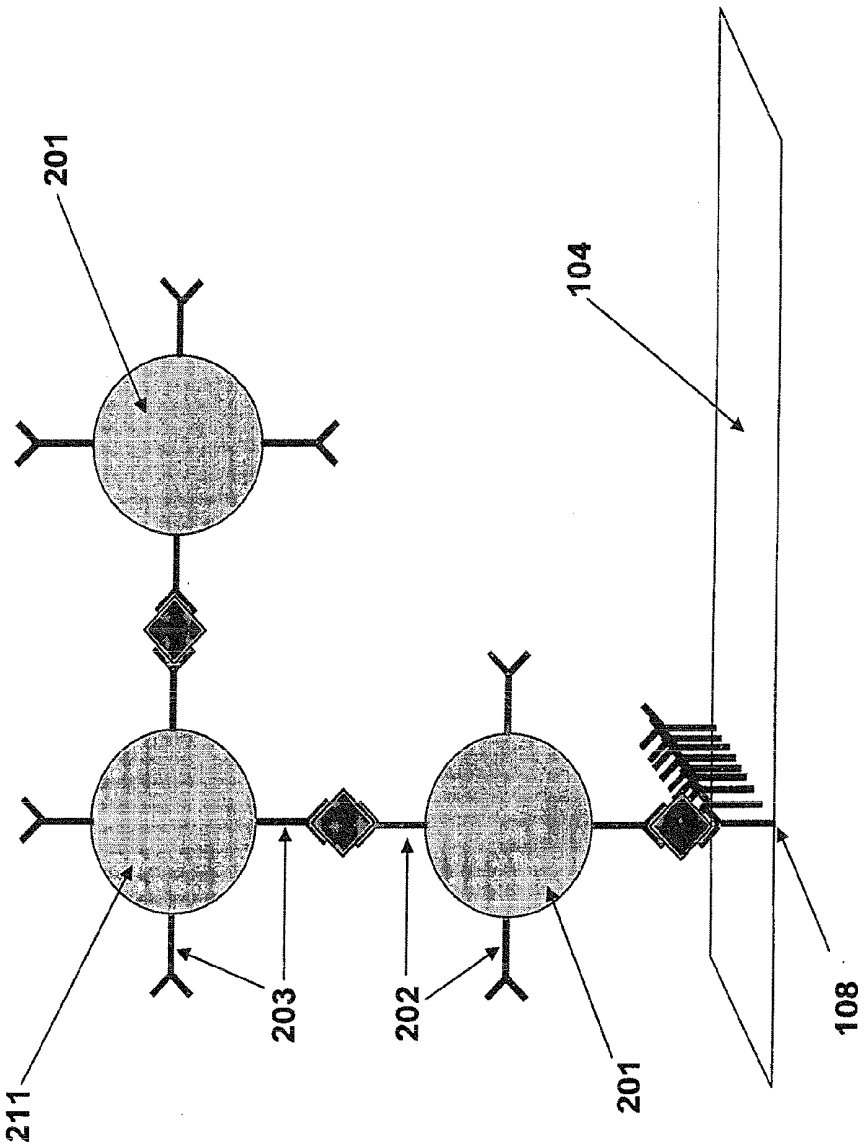


Figure 5

RAPID IMMUNOCHROMATOGRAPHIC DETECTION BY AMPLIFICATION OF THE COLLOIDAL GOLD SIGNAL

[0001] The present invention relates in general to the field of diagnostics, namely to a device for the detection of a target in a sample. More precisely, the present invention relates to a rapid immunochromatographic test device especially suitable for ultra-sensitivity detection of an antibody and/or antigen in a sample using double sandwich immunoassay detection for sensitivity enhancement by signal amplification. The present invention further refers to a method for the production of the test device, to the uses of the test device for the early detection of disease infection such as HIV in a sample, as well as to a kit comprising the test device.

BACKGROUND OF THE INVENTION

[0002] In recent years the in vitro diagnostics (IVD) industry has made enormous efforts to develop immunochromatographic tests. Such tests have found applications in both clinical and non-clinical fields[1]. A clinical utility of this test format has been shown for more than 150 different analytes, and many of them are target now of commercially available diagnostic products[3]. The wide range of applications for such devices has been reviewed[1,2].

[0003] Rapid immunochromatographic test devices, e.g. in the form of a test strip, are made up of a number of components (FIG. **1***a*). Such a test strip **101** commonly includes a sample pad **102**, a conjugate pad **103**, a membrane **104**, e.g. a nitrocellulose membrane, and an absorbent pad **105**. The membrane **104** is usually attached by means of an adhesive **106** to a supporting backing **107**, e.g. made of plastic. In practice, the user dispense a patient sample (usually urine or whole blood) onto the sample pad **102**. The sample then flows through the sample pad **102** into the conjugate pad **103**, where it mixes with and releases the detector reagent. This mixture then flows across the membrane **104**, where it binds with the test and control reagents located in the capture test zone **108** (sample zone) and negative control zone **109**, respectively. When the mixture binds to the reagent that forms the test line, a positive result is indicated. The colour intensity of the test line is proportional to the concentration of analyte in the sample. Excess sample that flows beyond the test and control zones **108**, **109** is taken up in the absorbent pad **105**.

[0004] Rapid immunochromatographic test devices for diagnostic purposes are easy to operate and thus do not only contribute to the comfort of professional users, e.g. medical stuff, but also allow the operation by non-professionals users, e.g. most patients.

[0005] However, despite the wide use of rapid immunochromatographic test devices, their suitability is still limited with regard to certain applications. Urine, for example, contains very low levels of IgG, frequently around 1 mg/l. Therefore, the detection of antibodies, e.g. directed to HIV or HCV, require very sensitive techniques. To date, the tests for antibodies in urine samples are based on ELISA and Western blot techniques, which are labour-intensive, time-consuming and need to be carried out by qualified persons. Efforts are being made to develop simple and/or rapid tests for the detection of antibody to HIV in urine specimens[4].

[0006] Oral fluid specimens consist often of saliva, which predominantly contains IgA class antibody, and oral mucosal transudates, which mostly contain IgG, and therefore also have much lower levels of IgG than serum. The levels of IgG normally found in oral fluid specimens (approximately 15 mg/l) are, however, higher than in urine specimens and innovative simple and rapid technology that has been shown to be effective for whole blood, serum and plasma, e.g. lateral flow through a chromatographic membrane, has been developed for use with these specimens[4].

[0007] Human chorionic gonadotropin (hCG) is a glycopeptide hormone produced by the placenta during pregnancy. The appearance and rapid increase in the concentration of hCG in the subject's urine makes it a good marker for confirming pregnancy. The concentration of hCG in urine increases steadily to a circulation peak of as much as 50,000 mIU/ml between the eighth and eleventh weeks.

[0008] Urine hCG levels during pregnancy are estimated to be:

[0009] 1. 10-30 mIU/ml 7-10 days post conception.

[0010] 2. 37,000-50,000 mIU/ml 8-11 weeks after last menstrual period.

[0011] 3. <5 mIU/ml Healthy men or non-pregnant women.

[0012] In the prior art the hCG test is a chromatographic immunoassay which uses specific antibodies to selectively identify hCG in urine with a high degree of sensitivity. Elevated levels of hCG as low as 20 mIU/ml can be detected within 3 minutes.

[0013] So far there are several tests used to detect the presence of hepatitis B antibodies. In addition, there are also several tests in the prior art that detect the presence of viral antigens.

[0014] The hepatitis B surface antibody (anti-HBs) detection is one of the most common test. Its presence indicates previous exposure to HBV, but the virus is no longer present and the person cannot pass on the virus to others. The antibody also protects the body from future HBV infection. In addition to exposure to HBV, the antibodies can also be acquired from successful vaccination. This test is done to determine the need for vaccination (if anti-HBs is absent), or following the completion of vaccination against the disease, or following an active infection. Hepatitis B surface antigen (HBsAg) is a protein antigen produced by HBV. This antigen is the earliest indicator of acute hepatitis B and frequently identifies infected people before symptoms appear. HBsAg disappears from the blood during the recovery period. In some people (particularly those infected as children or those with a weak immune system, such as those with AIDS), chronic infection with HBV may occur and HBsAg remains positive.

[0015] Further, testing for HIV is an essential component in the diagnosis and treatment of persons infected with the virus, in screening of blood for transfusion, in surveillance and in HIV/AIDS related research. Thus accurate and cost-effective testing is of great importance in combating the spread of HIV. It is imperative that tests for the diagnosis of HIV infection be as accurate as possible, given the serious ethical, legal and social issues that accompany HIV infection.

[0016] The number of people living with HIV has now risen to reach its highest level ever: close to 40 million people are living with the virus and close to 5 million people were newly infected with HIV in 2004 alone. Worldwide, the AIDS epidemic killed over 3 million people last year alone (Source: UNAIDS). Furthermore, only one in five people needing HIV prevention worldwide have access to basic prevention services and only one in ten people living with HIV has been tested for the virus.

[0017] The HI virus is most easily transmitted to others during the initial period of acute HIV infection, when the viral load (quantity of HIV RNA in the blood) is especially high and when people are not aware of being contaminated by the virus. Most HIV infections are transmitted at this stage, called primary infection. Earlier detection using ultra sensitive tests avoids missing primary infections, enabling immediate precautionary measures to be taken to help prevent the risk of HIV transmission to a non-infected partner, to an unborn child, or through blood donations or direct blood contact. Earlier detection of HIV infection also ensures the implementation of early antiretroviral therapy (ART) to slow down the progression of HIV infection, thereby improving patient care and quality of life.

[0018] The diagnosis of HIV infection is usually made on the basis of the detection of HIV antibodies and/or antigen. The diagnosis of an HIV infection can be made indirectly, i.e. through the demonstration of virus-specific antibodies. Besides such indirect diagnosis based on detection of antibodies, a direct diagnosis of HIV infection is also possible: either through the demonstration of infectious virus (using cell culture), viral antigens (p24 antigen ELISA) or viral nucleic acid (i.e. viral genome); the latter is also termed nucleic acid testing (NAT).

[0019] One important problem of HIV antibody testing is the so-called "diagnostic window". This is the time period that elapses between the time of acquisition of HIV infection until detectable levels of antibodies are present. The switch from antibody-negative to antibody-positive is called "seroconversion".

[0020] The most widely used screening tests are ELISAs as they are the most appropriate for screening large numbers of specimens on a daily basis, e.g. blood donations. The earliest assays used purified HIV lysates (1st generation assays). Improved assays based on recombinant proteins and/or synthetic peptides, which also enabled the production of combined HIV-1/HIV-2 assays, became rapidly available (2nd generation assays). The so-called 3rd generation or antigen-sandwich assays, which use labelled antigens as conjugate, are more sensitive and have reduced the diagnostic window period considerably[5,6].

[0021] Thus, there is need in the prior art to provide a rapid immunochromatographic test device suitable for the ultra-sensitive detection of target in a sample.

[0022] It is an object of the present invention to overcome the problems especially with regard to the applicability of rapid immunochromatographic test devices for the detection of hCG, HBsAG, anti-HBs, IgG, e.g. HIV antibodies, in urine, blood, serum or saliva by enhanced sensitivity.

[0023] It is therefore an object to enhance the sensitivity of the rapid immunochromatographic detection system. Thus, it is an object of the present invention to overcome the drawbacks of the prior art and to provide especially a simple and rapid test device for the ultra-sensitive antibody and/or antigen detection by signal development and signal amplification suitable to be employed for the early detection of disease infections in a sample.

SUMMARY OF THE INVENTION

[0024] In one embodiment the present invention concerns a rapid immunochromatographic test device for the detection of a target in a sample, comprising

[0025] a) a first gold conjugate releasing pad, comprising colloidal gold conjugated with a first antibody or antigen, and

[0026] b) a second gold conjugate releasing pad, comprising colloidal gold conjugated with a second antibody or antigen;

[0027] wherein both releasing pads are located at different positions within the test device.

[0028] In a further embodiment the present invention concerns a method for the production of a device according to the present invention comprising the steps of

[0029] a) preparing a colloidal gold solution;

[0030] b) preparing a conjugation buffer;

[0031] c) partitioning the conjugation buffer by dividing it into a first and a second flask;

[0032] d) adding an antibody according to the present invention to the conjugation buffer in the first flask;

[0033] e) adding an antibody according to the present invention to the conjugation buffer in the second flask, wherein said antibody differs from the antibody used in step d);

[0034] f) adding colloidal gold solution into each flask;

[0035] g) adding stabilizing buffer to each flask;

[0036] h) concentrating each conjugate;

[0037] i) adding a surfactant to the first conjugate and soaking glass fibre sheet conjugate pad into the conjugate;

[0038] j) soaking another glass fibre sheet conjugate pad into the second conjugate;

[0039] k) printing sample and control lines onto the membrane;

[0040] l) laminating cards using the first gold conjugate; and

[0041] m) cutting cards into strips.

[0042] In another embodiment the present invention relates to the use of a device according to the present invention for the detection of a disease in at least one sample.

[0043] In a further embodiment the present invention refers to a kit for detection of a disease comprising the device according to the present invention and a manual.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

[0044] Before the present invention is described in more detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

[0045] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step.

[0046] Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their

entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0047] As outlined above there is a need in the prior art to provide a new test device suitable for the early detection of a disease infection in at least one sample. Further, there is a need in the prior art to provide a new method for rapid immunochromatographic detection of a target in a sample for the detection of a disease or a specific condition such as pregnancy in a subject. In addition, there is also a need in the art for devices suitable for simple, rapid and ultra-sensitive detection of an antigen and/or antibody, which devices having a higher sensitivity than devices from the prior art.

[0048] In a first aspect the present invention provides a rapid immunochromatographic test device for the detection of a target in a sample, comprising

[0049] a) a first gold conjugate releasing pad, comprising colloidal gold conjugated with a first antibody or antigen, and

[0050] b) a second gold conjugate releasing pad, comprising colloidal gold conjugated with a second antibody or antigen;

[0051] wherein both releasing pads are located at different positions within the test device.

[0052] The first colloidal gold conjugated with a first antibody or antigen captures the target in the sample and forms a complex "target-first colloidal conjugate". Preferably this target in the sample is an antigen and/or antibody.

[0053] In a embodiment of the device according to the present invention the first gold conjugate releasing pad comprises a gold conjugate that is conjugated with a first specific antibody or antigen to capture the target analyte from the first site. The second gold conjugate releasing pad comprises a gold conjugated with a second specific antibody or antigen to capture the target analyte from the second site. The last mentioned conjugated antibody or antigen is the same antibody or antigen that is immobilized onto the nitrocellulose membrane.

[0054] In one preferred embodiment of the device according to the present invention the first gold conjugate releasing pad comprises a gold conjugate **201** that is conjugated with a first specific antibody **202** or antigen to capture the target analyte from the first site **202'**. The second gold conjugate releasing pad comprises a gold **211** conjugated with a second specific antibody **203** or antigen to capture the target analyte from the second site **203'**. The last mentioned conjugated antibody **203** or antigen is the same antibody or antigen that is immobilized onto the nitrocellulose membrane (FIG. **3**).

[0055] In another embodiment of the device according to the present invention the device comprises a test strip comprising

[0056] a) a sample pad,

[0057] b) a conjugate pad comprising the first gold conjugate pad,

[0058] c) a conjugate pad comprising the second gold conjugate pad,

[0059] d) a membrane comprising a capture test zone and a negative control zone, and

[0060] e) an absorbent pad.

[0061] In a preferred embodiment of the device according to the present invention the capture test zone comprises the second antibody or antigen. The antibody or antigen within the test zone capture the target from a site that differs from that site captured by the first antibody conjugated with the first colloidal gold, why both antibodies differ from each other.

[0062] In another preferred embodiment the second specific antibody or antigen is immobilized within the test zone. The complex "target-first colloidal gold conjugate" will be captured by this second antibody or antigen and therefore kept within the test zone to form the sandwich detection (FIGS. **3** and **4**). Then, the second gold conjugate releasing pad will release its gold conjugated with the second specific antibody or antigen to capture the target analyte from the second site. The second conjugate would bind with the first conjugate from the side of the target (FIGS. **3** and **4**). At the same time, the other free sides of the target will be able to link with their specific antibody or antigen to form more and more branched bonds that propagate the accumulation of colloidal gold particles onto the capturing/sample line. This propagation and accumulation of colloidal gold signal will amplify the signal and highly increase the sensitivity. This will enable us to detect very low concentrations that are not detectable using the same technique without signal amplification.

[0063] In one embodiment of the device according to the present invention the membrane is attached by means of an adhesive to a supporting backing. Preferably an acrylic pressure sensitive adhesive as known in the art is used.

[0064] In another embodiment of the device according to the present invention the first and second gold conjugate pad are laminated between the sample pad and the membrane, wherein the two gold conjugates are separated by a divider.

[0065] In a preferred embodiment of the device according to the present invention the first **103.1** and second gold conjugate pad **103.2** are laminated between the sample pad **102** and the membrane **104**, wherein the two gold conjugates are separated by a divider **110** (FIG. **1***b*). Preferably, the divider is an inert divider, more preferably the divider is a plastic divider

[0066] In another embodiment, the device according to the present invention the first gold conjugate pad is attached between the sample pad and the membrane while the second gold conjugate pad is within the upper part of the plastic housing to be released after sample application onto the nitrocellulose membrane directly.

[0067] In one preferred embodiment of the device according to the present invention the supporting backing is a plastic backing.

[0068] In another preferred embodiment of the device according to the present invention the membrane is nitrocellulose membrane.

[0069] In one embodiment of the device according to the present invention the first or second antibody is selected from the group comprising mouse anti-HIV p24, mouse anti-HBsAg, anti-hlgG, anti-Lipoarabinomannan, anti-H.Pylori antigen, anti-Leishmania antigen, anti-Pneumonia antigen, anti-Malaria antigen, anti-Chlamydia antigen, anti-Toxoplasma antigen, anti-Schistosoma antigen, HIV 1 antibody, and HIV 2 antibody.

[0070] In a preferred embodiment of the device according to the present the first or second antibody is a monoclonal or polyclonal antibody. Preferably the first and second antibodies are two different monoclonal antibodies that recognize the target from two different sites.

[0071] In another embodiment of the device according to the present invention the first antigen is selected from the group comprising conjugate of HIV antigen, conjugate of

hepatitis C antigen, HIV 1 antigen, HIV 2 antigen, Lipoarabinomannan, H.Pylori antigen, Toxoplasma antigen.

[0072] In a further embodiment of the device according to the present invention the control zone comprises a non-specific capturing antibody and/or a non-specific antibody capturing protein.

[0073] In one preferred embodiment of the device according to the present invention the non-specific antibody is selected from the group consisting of anti-mouse IgG, anti-rabbit IgG, anti-goat IgG, anti-donkey IgG, Anti-sheep IgG, anti-HIV p24, anti-Lipoarabinomannan, anti-H.Pylori antigen, anti-Leishmania antigen, anti-Pneumonia antigen, anti-Malaria antigen, anti-Chlamydia antigen, anti-Toxoplasma antigen, anti-Schistosoma antigen, HIV 1 antibody, and HIV 2 antibody.

[0074] In another preferred embodiment of the device according to the present invention the non-specific capturing protein is either Protein A or Protein G.

[0075] In a preferred embodiment of the device according to the present invention the device comprises a housing comprising at least one test strip according to the present invention.

[0076] In another preferred embodiment of the device according to the present invention the housing comprises two, three, four, five, six, seven, eight, nine, or ten test strips. Preferably the housing comprises two, three, four, or five test strips, more preferably the housing comprises two or three test strips.

[0077] In one preferred embodiment of the device according to the present invention each test strip contains at least two antibodies or antigens, or at least one antibody and one antigen, wherein one of these antibodies or antigens is immobilized onto the membrane and the other one is conjugated with the first colloidal gold. In case of two antibodies, they have to be different to capture the target from two different sites.

[0078] In another aspect the present invention concerns a method for the production of a device according to the present invention, comprising the steps of

[0079] n) preparing a colloidal gold solution;

[0080] o) preparing a conjugation buffer;

[0081] p) partitioning the conjugation buffer by dividing it into a first and a second flask;

[0082] q) adding an antibody according to the present invention to the conjugation buffer in the first flask;

[0083] r) adding an antibody according to the present invention to the conjugation buffer in the second flask, wherein said antibody differs from the antibody used in step d);

[0084] s) adding colloidal gold solution into each flask;

[0085] t) adding stabilizing buffer to each flask;

[0086] u) concentrating each conjugate;

[0087] v) adding a surfactant to the first conjugate and soaking glass fibre sheet conjugate pad into the conjugate;

[0088] w) soaking another glass fibre sheet conjugate pad into the second conjugate;

[0089] x) printing sample and control lines onto the membrane;

[0090] y) laminating cards using the first gold conjugate; and

[0091] z) cutting cards into strips.

[0092] In another aspect the present invention relates to the use of a device according to the present invention for the detection of a disease in at least one sample.

[0093] In one preferred embodiment of the use according to the present invention the antibody in one sample (e.g. specimen) and the antigen in another sample (e.g. specimen) is detected. For example, in the case two test strips are used, Lipoarabinomannan-antigen can be detected in urine, while anti-lipoarabinomannan is detected in serum.

[0094] In another preferred embodiment of the use according to the present invention the antibody and antigen are detected in the same sample (specimen). For example, HIV antibodies and the HIV p24 antigen are detected in the same serum sample (specimen) using a device of two different strips.

[0095] In one embodiment of the use of the device according to the present invention the sample is obtained from a human.

[0096] In one preferred embodiment of the use of the device according to the present invention the sample is selected from the group comprising of whole blood, serum, plasma, saliva, and urine.

[0097] In another preferred embodiment of the use of the device according to the present invention the disease detected in said sample is selected from the group consisting of HIV, Hepatitis A, Hepatitis B, Hepatitis C, H.Pylori, Leishmania, Schistosomiasis, Malaria, Pneumonia, Toxoplasmosis, Tubercolosis and Chlamydial infection.

[0098] In a further aspect the present invention refers to a kit for detection of a disease comprising the device according to the present invention and a manual.

[0099] In one preferred embodiment of the kit according to the present invention the kit further comprises an assay buffer. The assay buffer can be any buffer known in the art suitable for the use of whole blood samples. Preferably in the case of whole blood samples Tris buffer is used, more preferably 0.1M Tris buffer having a pH of 7.5 and comprising a preservative. Any preservative known by a person skilled in the art can be used, preferably sodium azide and even more preferably 0.01M sodium azide is used.

[0100] In another embodiment the present invention relates to the use of the method for diagnosing and monitoring a disease or a specific condition of a subject by detecting a target in a sample.

[0101] The following example illustrate the present invention without, however, limiting the same thereto.

BRIEF DESCRIPTION OF THE DRAWING

[0102] FIG. **1***a*: shows top and side views of a typical rapid-flow immunochromatographic test device known in the art in the form of a test strip **101** comprising a sample pad **102**, a conjugate pad **103**, a membrane **104**; an absorbent pad **105**, an adhesive **106**, a supporting backing **107**, a test zone **108**, and a control zone **109**.

[0103] FIG. **1***b*: shows top and side views of a preferred embodiment of a rapid-flow immunochromatographic test device according to the present invention in the form of a test strip **101** comprising a sample pad **102**, a first conjugate pad **103.1**, a second conjugate pad **103.2**, a membrane **104**, an absorbent pad **105**, an adhesive **106**, a supporting backing **107**, a test zone **108**, a control zone **109**, and the conjugates divider **110**.

[0104] FIG. **2**: shows the schematically view of a preferred embodiment of the first and second colloidal gold according to the present invention, wherein the first colloidal gold **201** is conjugated with a first specific antibody **202** and wherein the second colloidal gold **211** is conjugated with a second spe-

cific antibody **203**. In addition, the target is shown having two sides **202'** and **203'**. The first side **202'** of the target is captured by the first antibody **202** of the first gold conjugate **201** and the second side **203'** of the target is captured by the second antibody **203** conjugated with the second gold conjugate **211**.

[0105] FIG. **3**: shows a simplified scheme of a preferred embodiment of the test device according to the present invention. It shows the test zone **108** of the membrane **104** on the test strip **101**, wherein the second specific antibody **203** or antigen is immobilized to the test zone **108**.

[0106] FIG. **4**: shows the main principle of a preferred embodiment of the signal development according to the present invention. By the sample flow within the rapid immunochromatographic test device the target in the sample will be captured by the first specific antibody **202** or antigen of the first colloidal gold **201** to form the complex "target-first colloidal gold". This complex flows to the test zone **108**, where it will be captured by the second specific antibody **203** or antigen that is immobilized onto the membrane **104** to form a sandwich detection.

[0107] FIG. **5**: shows the main principle of a preferred embodiment of the signal amplification and multiplication according to the present invention. By the sample flow within the rapid immunochromatographic test device the target in the sample will be captured by the first specific antibody **202** or antigen that is conjugated to the first colloidal gold **201** to form the complex "target-first colloidal gold". This complex flows to the test zone **108**, where it will be captured by the second specific antibody **203** or antigen that is immobilized onto the membrane **104** of the test zone **108**. Then, the second colloidal gold **211** conjugated with the second specific antibody **203** or antigen will be released and will bind to the target as well as to the first colloidal gold conjugate **201** and enhance the signal by forming a double sandwich.

EXAMPLES

[0108] The following examples illustrate the present invention without, however, limiting the same thereto.

Example 1

Preparation of an Preferred Embodiment of a Test Device According to the Present Invention

[0109] a) prepare 1% aqueous solution of tetrachloroauric acid at room temperature;

[0110] b) prepare 4% trisodium citrate aqueous solution at room temperature;

[0111] c) prepare 0.05 M Potassium Carbonate aqueous solution at room temperature;

[0112] d) prepare 600 ml of phosphate stabilizing buffer of pH 7.4, containing BSA, Tween 20, Sucrose, polyvinylpurrolidone and a preservative, e.g. sodium azide, at room temperature;

[0113] e) prepare colloidal gold solution by reduction of 1.7 ml boiling tetrachloroauric acid solution (after dilution into 100 ml) using 1 ml trisodium citrate solution and let it takes the room temperature;

[0114] f) dilute the colloidal gold solution as 1:1 using distilled water. Adjust the pH to 7.4 using potassium carbonate solution at room temperature;

[0115] g) prepare 200 ml of phosphate conjugation buffer of pH 7.4 at room temperature;

[0116] h) partition the 200 ml conjugation buffer by dividing it into two flasks (100 ml of each);

[0117] i) add 1.0 mg of aqueous antibody (e.g. anti-p24 1^{st} clone) to the conjugation buffer in the first flask with stirring at room temperature;

[0118] j) add 1.0 mg of aqueous antibody (e.g. anti-p24 2^{nd} clone) to the conjugation buffer in the second flask with stirring at room temperature;

[0119] k) add 100 ml colloidal gold solution into each flask with stirring at room temperature;

[0120] l) after about 45 minutes; add 200 ml of stabilizing buffer to each flask;

[0121] m) after about 20 minutes; concentrate each conjugate by cooled (temperature around 15° C.) high speed centrifugation (10,000 rpm for one hour);

[0122] n) discard the supernatant and re-suspend the concentrated conjugates using the stabilising buffer at room temperature;

[0123] o) adjust the concentration for each of the two conjugates to $O.D._{520}=2.0$;

[0124] p) add 0.1 ml of Tween 20 to the first conjugate and soak glass fibre sheet conjugate pad into the conjugate, then heat dry at temperature around 50° C.; and

[0125] q) soak another glass fiber sheet conjugate pad into the second conjugate, then heat dry at temperature around 50° C.

[0126] (*In case of antibodies/antigens and their specific antigens/antibodies there is no need for these steps of bovine serum albumin or any other protein labelling. ** Other proteins or peptides could be used other than bovine serum albumin).

[0127] Additionally, print sample (e.g. anti-p24, 2^{nd} clone) and control lines (e.g. anti-mouse IgG) onto nitrocellulose membrane, then heat dry at temperature around 50° C.

[0128] Finally, laminate cards according to the following procedure:

A. In Case of Conjugate Releasing Site Laminated within the Upper Side of the Device Plastic Housing

[0129] Lamination of cards using the first gold conjugate. Laminate card components onto the backing material with the sequence:

[0130] 1. laminate the nitrocellulose membrane nearly in the middle of the card;

[0131] 2. laminate the absorbent pad in the end of the card (overlaps from the nitrocellulose membrane side);

[0132] 3. laminate the first conjugate pad in the other side of the nitrocellulose membrane; and

[0133] 4. laminate the sample pad.

B. In Case of Conjugate Releasing Site Laminated onto the Test Strip Itself Separated from the First Conjugate by a Divider

[0134] Laminate card components onto the backing material with the sequence (see FIG. **1***b*):

[0135] 1 laminate the nitrocellulose membrane nearly in the middle of the card;

[0136] 2. laminate the absorbent pad in the end of the card (overlaps from the nitrocellulose membrane side);

[0137] 3. laminate the first conjugate pad in the other side of the nitrocellulose membrane;

[0138] 4. laminate the plastic divider onto the first conjugate (overlaps from the nitrocellulose membrane side);

[0139] 5. laminate the second conjugate pad onto the divider (overlaps from the nitrocellulose membrane side);

[0140] 6. laminate the sample pad onto the other end of the card, the sample pad will overlaps with the two conjugate pads; and

[0141] 7. then cut cards into strips.

C. Alternatively

[0142] Lamination of the second gold conjugate could be applied within the plastic housing itself to ensure that the two conjugates will not propagate before release from the releasing pad and so stick within the releasing pad.

Example 2

Hepatitis B Surface Antigen (HBsAg) Detection System

[0143] The first gold conjugate pad contains a conjugate of colloidal gold with a first mouse monoclonal anti-HBsAg, and the second gold conjugate pad contains a conjugate of colloidal gold with a second mouse monoclonal anti-HBsAg. The first conjugate releasing pad **103.1** is laminated on the test strip between the sample pad and the nitrocellulose membrane **104** while the second **103.2** is above the first pad separated by a divider **110** to be released directly toward the nitrocellulose membrane **104** without flow through the first conjugate pad to avoid interact with the first conjugate before reaching the membrane (FIG. **1***b*).

[0144] The second conjugate releasing site could be laminated within the upper side of the device plastic housing.

[0145] The sample line **108** is the second mouse monoclonal anti-HBsAg immobilized onto the nitrocellulose membrane **104**. The control line **109** is anti-mouse IgG. Sample **108** and control lines **109** turn into purple color in case of HBsAg availability in the sample; only the control line **109** turns into purple color in case of HBsAg free sample, see FIG. **1**B.

[0146] The commercially available rapid tests sensitivity for Hepatitis B surface antigen is within the range 500-1000 pg/ml while according to this system it is so simple to detect less than 200 pg/ml.

Example 3

Human Immunodeficiency Virus (HIV) Antibodies Detection System

[0147] The first gold conjugate pad contains a conjugate of colloidal gold with a first mouse anti-human Immunoglubulin G (anti-hIgG), and the second gold conjugate pad contains a conjugate of colloidal gold with HIV p160. The first conjugate releasing pad **103.1** is laminated on the test strip between the sample pad and the nitrocellulose membrane while the second **103.2** is above the first pad separated by a divider **110** to be released directly toward the nitrocellulose membrane without flow through the first conjugate pad to avoid interact with the first conjugate before reaching the membrane (FIG. **1***b*).

[0148] The second conjugate releasing site could be laminated within the upper side of the device plastic housing.

[0149] The sample line **108** is HIV p160 antigen immobilized onto the nitrocellulose membrane **104**. The control line **109** is anti-mouse IgG. Sample **108** and control lines **109** turn into purple colour in case of HIV antibodies availability in the sample; only the control line **109** turns into purple colour in case of HIV antibodies free sample, see FIG. **1**B.

[0150] According to this system it is so simple to detect very low titers of HIV antibodies.

[0151] The features disclosed in the foregoing description, in the claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realizing the invention in divers forms thereof.

REFERENCES

[0152] (1) J Chandler, N Robinson, and K Whiting, "Handling False Signals in Gold-Based Rapid Tests", IVD Technology 7, no. 2 (2001): 34-45; http://www.devicelink.com/ivdt/archive/01/03/002.html.

[0153] (2) J Chandler, T Gurmin, and N Robinson, "The Place of Gold in Rapid Tests", IVD Technology 6, no. 2 (2000): 37-49; http://www.devicelink.com/ivdt/archive/00/03/004.html

[0154] (3) T C Tisone et al., "Image Analysis for Rapid-Flow Diagnostics", IVD Technology 5, no. 5 (1999): 52-58; http://www.devicelink.com/ivdt/archive/99/09/010.html.

[0155] (4) Zaaijer, H. L., Exel-Oehlers, P. V., Kraaijeveld, T., Altena, E., Lelie, P. N. (1992) Early detection of antibodies to HIV-1 by third-generation assays. *Lancet* 340, 770-772.

[0156] (5) Constantine, N. T., van der Groen, G., Belsey, E. M., Tamashiro, H. (1994) Sensitivity of HIV-antibody assays determined by seroconversion panels. *AIDS* 8, 1715-1720.

[0157] (6) Satten, G. A., Busch, M. P., et al. (1997) Effect of transmission route on window period estimates. Fourth Conference on Retroviruses and Opportunistic Infections, Washington D.C., Abstract 122.

[0158] (7) WHO. Rapid HIV tests: Guidelines for use in HIV testing and counseling services in resource-constrained settings. Geneva 2004. http://www.who.int/hiv/pub/vct/rapidhivtests/en/

[0159] (8) Holodniy M, et al. (1991) Reduction in plasma human immunodeficiency virus ribonucleic acid following dideoxynucleoside therapy as determined by the polymerase chain reaction. *J. Clin. Invest* 88, 1755-1759.

[0160] (9) Katzenstein D. A., et al. (1994) Quantitation of human immunodeficiency virus by culture and polymerase chain reaction in response to didanosine after long-term therapy with zidovudine. *J. Infect. Dis.* 169, 416-419.

[0161] (10) Jackson J B, et al. (1998) Practical diagnostic testing for human immunodeficiency virus. *Clin. Microb. Rev.* 1, 124-138.

[0162] (11) Goudsmit J, et al. (1986) Expression of human Immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet* 2, 177-180.

[0163] (12) Aubuchon, J. P., Birkmeyer, J. D., Busch, M. P. (1997) Cost-effectiveness of expanded human immunodeficiency virus-testing protocols for donated blood. *Transfusion* 45, 45-51.

[0164] (13) Ward, J. M., Holmberg, S. D., Allen, J. R., Cohn, D. L., et al. (1988) Transmission of human immunodeficiency virus (HIV) by blood transfusion screened as negative for HIV antibody. *N. Engl. J. Med.* 8, 473-478.

[0165] (14) Alter, H. J., et al. (1990) Prevalence of human immunodeficiency virus type 1 p24 antigen in U.S. blood donors—an assessment of the efficacy of testing in donor screening. *N. Engl. J. Med.* 323, 1312-1317.

[0166] (15) Mayers, D. L. (1998) Drug-resistant HIV-1. The virus strikes back. *JAMA* 279, 2000-2002.

[0167] (16) Stephenson, J. (2002) Cheaper HIV drugs for poor nations bring a new challenge: monitoring treatment. *JAMA* 288, 2.

[0168] (17) WHO. HIV assays: Operational characteristics (Phase 1). Report 15/antigen/antibody ELISAs. Geneva 2004. http://www.who.int/diagnostics_laboratory/evaluations/hiv/en/

[0169] (18) WHO. HIV assays: Operational characteristics (Phase 1). Report 14/simple/rapid tests. Geneva 2003. http://www.who.int/diagnostics_laboratory/evaluations/hiv/en/

[0170] (19) Meier, T, et al. (2001) Evidence for a diagnostic window in fourth generation assays for HIV. *J. Clin. Virol.* 23, 113-116.

1. A rapid immunochromatographic test device for the detection of a target in a sample, comprising

(a) a first gold conjugate releasing pad, comprising colloidal gold conjugated with a first specific antibody or antigen, and

(b) a second gold conjugate releasing pad, comprising colloidal gold conjugated with a second specific antibody or antigen;

wherein both releasing pads are located at different positions within the test device.

2. The device according to claim **1**, wherein said device comprises a test strip comprising

a) a sample pad,

b) a conjugate pad comprising said first gold conjugate pad,

c) a conjugate pad comprising said second gold conjugate pad,

d) a membrane comprising a capture test zone and a negative control zone, and

e) an absorbent pad.

3. The device according to claim **2**, wherein said capture test zone comprises said second antibody or antigen.

4. The device according to claim **2**, wherein said membrane is attached by means of an adhesive to a supporting backing.

5. The device according to claim **2**, wherein said first and second gold conjugate pad are laminated between the sample pad and the membrane, wherein said two gold conjugate pads are separated by a divider.

6. The device according to claim **2**, said first gold conjugate pad is attached between the sample pad and the membrane while the second gold conjugate pad is within the upper part of the plastic housing.

7. The device according to claim **4**, wherein said supporting backing is a plastic backing.

8. The device according to claim **2**, wherein said membrane is nitrocellulose membrane.

9. The device according to claim **1**, wherein said first or second antibody is selected from the group comprising mouse anti-HIV p24, mouse anti-HBsAg, anti-hlgG, anti-Lipoarabinomannan, anti-H.Pylori antigen, anti-Leishmania antigen, anti-Pneumonia antigen, anti-Malaria antigen, anti-Chlamydia antigen, anti-Toxoplasma antigen, anti-Schistosoma antigen, HIV 1 antibody, and HIV 2 antibody.

10. The device according to claim **1**, wherein said first antigen is selected from the group comprising conjugate of HIV antigen, conjugate of hepatitis C antigen, HIV 1 antigen (HIV p160), HIV 2 antigen (HIV p36), Hepatitis B antigen, Lipoarabinomannan, H.Pylori antigen, Toxoplasma antigen.

11. The device according to claim **2**, wherein said control zone comprises a non-specific capturing antibody and/or a non-specific antibody capturing protein.

12. The device according to claim **11**, wherein said non-specific antibody is selected from the group consisting of anti-mouse IgG, anti-rabbit IgG, anti-goat IgG, anti-donkey IgG, Anti-sheep IgG, anti-HIV p24, anti-Lipoarabinomannan, anti-H.Pylori antigen, anti-Leishmania antigen, anti-Pneumonia antigen, anti-Malaria antigen, anti-Chlamydia antigen, anti-Toxoplasma antigen, anti-Schistosoma antigen, HIV 1 antibody, and HIV 2 antibody.

13. The device according to claim **11**, wherein said non-specific capturing protein selected is either Protein A or Protein G.

14. The device according to claim **1**, comprising at least one test strip according to any of claims **2-13**.

15. A method for the production of a device according to claim **1**, comprising the steps of comprising the steps of

a. preparing a colloidal gold solution;

b. preparing a conjugation buffer;

c. partitioning the conjugation buffer by dividing it into a first and a second flask;

d. adding an antibody according to claim **9** to the conjugation buffer in the first flask;

e. adding an antibody according to claim **9** to the conjugation buffer in the second flask, wherein said antibody differs from the antibody used in step d);

f. adding colloidal gold solution into each flask;

g. adding stabilizing buffer to each flask;

h. concentrating each conjugate;

i. adding a surfactant to the first conjugate and soaking glass fibre sheet conjugate pad into the conjugate;

j. soaking another glass fibre sheet conjugate pad into the second conjugate;

k. printing sample and control lines onto the membrane (**104**),

l. laminating cards using the first gold conjugate; and

m. cutting cards into strips.

16. A method claim **1** for the detection of a disease in at least one sample, the method comprising

contacting the sample with the device according to claim **1**, and

checking the device for indication of presence of the target.

17. The method according to claim **16**, wherein said sample is obtained from a human.

18. The method according to claim **17**, wherein said sample is selected from the group comprising of whole blood, serum, plasma, saliva, and urine.

19. The method according to claim **16**, wherein said disease detected in said sample is selected from the group consisting of HIV, Hepatitis A, Hepatitis B, Hepatitis C, H.Pylori, Leishmania, Schistosomiasis, Malaria, Pneumonia, Toxoplasmosis, Tubercolosis and Chlamydia infection.

20. A kit for detection of a disease comprising the device according to claim **1** and instructions.

21. The kit according to claim **20** further comprising an assay buffer.

22. The kit according to claim **21**, wherein said assay buffer comprises a preservative.

* * * * *

专利名称(译)	通过扩增胶体金信号进行快速免疫色谱检测		
公开(公告)号	US20100047816A1	公开(公告)日	2010-02-25
申请号	US12/518752	申请日	2007-12-06
[标]申请(专利权)人(译)	约旦PHARM MFG		
申请(专利权)人(译)	约旦医药制造有限公司. TERRAMARK MARKENCREATION GMBH		
当前申请(专利权)人(译)	阿拉根生物技术有限公司		
[标]发明人	BADWAN ADNAN MOHAMMED MURSHED ABDEL QADER		
发明人	BADWAN, ADNAN MOHAMMED, MURSHED ABDEL-QADER		
IPC分类号	G01N33/53 C12M1/34 G01N33/566 G01N30/00		
CPC分类号	G01N33/558 G01N33/54306 Y02A50/58		
优先权	2006025529 2006-12-11 EP		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及适用于检测至少一种样品中的抗体和/或抗原的快速免疫色谱测试装置，所述装置用于检测样品中疾病的用途，所述装置的制备方法以及包含该装置的试剂盒装置。

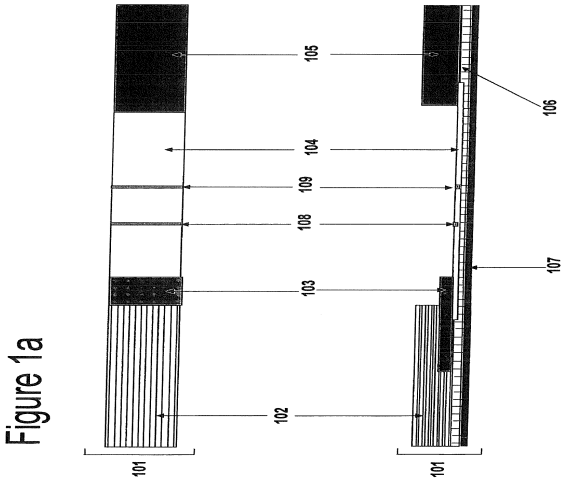


Figure 1a