(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 307 134 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
10.04.2013 Bulletin 2013/15

(21) Application number: **01957025.8**

(22) Date of filing: **10.08.2001**

(51) Int Cl.:
G01N 33/558 *(2006.01)* ***G01N 33/68*** *(2006.01)*
G01N 33/76 *(2006.01)*

(86) International application number:
PCT/KR2001/001365

(87) International publication number:
WO 2002/013685 (21.02.2002 Gazette 2002/08)

(54) **DIAGNOSTIC DEVICE FOR DISTINGUISHING BETWEEN NORMAL AND ECTOPIC PREGNANCY**

DIAGNOSEGERÄT ZUR UNTERSCHEIDUNG ZWISCHEN NORMALER UND EKTOPISCHER SCHWANGERSCHAFT

DISPOSITIF DE DIAGNOSTIC PERMETTANT DE FAIRE LA DISTINCTION ENTRE UNE GROSSESSE NORMALE ET UNE GROSSESSE EXTRA-UTERINE

(84) Designated Contracting States:
DE FR GB IT TR

(30) Priority: **12.08.2000 KR 2000046755**

(43) Date of publication of application:
07.05.2003 Bulletin 2003/19

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(56) References cited:
WO-A-95/06240 **WO-A-98/36278**
WO-A2-99/41584 **US-A- 4 016 250**
US-A- 5 185 270 **US-A- 5 236 846**
US-A- 5 786 220

- **COLE L A ET AL: "Urine hCG beta-subunit core fragment, a sensitive test for ectopic pregnancy." THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM. FEB 1994, vol. 78, no. 2, February 1994 (1994-02), pages 497-499, XP002410103 ISSN: 0021-972X**
- **COLE LAURENCE A: "Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites", CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY, WASHINGTON, DC, vol. 43, no. 12, 1 December 1997 (1997-12-01), pages 2233-2243, XP002292499, ISSN: 0009-9147**

EP 1 307 134 B1

Description

Technical Field

[0001] The present invention relates to one-step pregnancy diagnostic devices for distinguishing between a normal pregnancy and an ectopic pregnancy. More particularly, the present invention relates to diagnostic devices allowing rapid -and accurate determination of a normal pregnancy and an ectopic pregnancy at an early stage by immunologically detecting morphological differences between the human chorionic gonadotropin (hCG) and modified forms thereof, which are secreted into the body fluid of a pregnant female.

Background Art

[0002] An ectopic pregnancy refers to a pregnancy in which the fertilized egg is implanted outside of a normal uterine endometrial lining, and classified as a tubal pregnancy, a cervical pregnancy, an ovarian pregnancy, a peritoneal pregnancy and the like, depending on the site of implantation. More than 95% of ectopic pregnancy events are tubal pregnancy, which is thus being used as general designation for the ectopic pregnancy. The etiology of the ectopic pregnancy includes previous tubal ligation (contraception), PIID(Pelvic Inflammatory Disease), administration of ovulation controlling formulations and STD(SexuaUy Transmitted Disease), and the incidence of the ectopic pregnancy is quite high as one per about 64~241 pregnant females.

[0003] The ectopic pregnancy is one of the most frequent obstetric and gynecologic emergencies, and its incidence is increasing year by year. Observable symptoms of tubal rupture due to the ectopic pregnancy include lower abdominal pain, amenorrhea or vaginal bleeding, dizziness due to blood pressure drop, nausea and vomiting due to gastrointenstinal symptoms. Untreated tubal rupture may lead to intraperitoneal bleeding, which is emerging as the biggest cause of the death of pregnant females at early stages of pregnancy. Thus, if the ectopic pregnancy is not discovered in the early stages, tubal rupture and subsequent development to shock from bleeding may lead to death of the patient. However, early diagnosis of the ectopic pregnancy has faced with difficulties because there has not been found specific symptoms and diagnostic methods that can be applied specifically to the ectopic pregnancy. Under these circumstances, the present inventors have conducted an extensive study on the assumption that early determination of the ectopic pregnancy can reduce the maternity mortality and resolve the anxiety of females with normal pregnancy about ectopic pregnancy, and have now found methods and devices for an early diagnosis of the ectopic pregnancy.

[0004] Human chorionic gonadotropin (hereinafter, referred to as "hCG") is produced in syncytiotrophoblast of the placenta and induces a constant production of progesterone in the early stage of pregnancy to maintain the implantation until the 10^{th} week of pregnancy, when the placenta becomes completely functional. It also stimulates the production of testosterone in the fetal testis, plays an important role in the differentiation of internal genitalia in a male embryo, and has other various functions including the stimulation of maternal thyroid. It is detected in the blood and urine at a level of about 25mIU/ml at about 8 days after conception (or fertilization). Then, its concentration rises rapidly at a constant speed from the 5^{th} week of pregnancy such that the concentration reaches about 1 IU/ml at the 6^{th} week of pregnancy, and about 100~120 IU/ml at the 10^{th} week of pregnancy. From that point, the concentration decreases over the 10^{th} to the 18^{th} week of pregnancy to reach about 20 IU/ml at the 20^{th} week of pregnancy, after which the concentration is maintained constant. The hCG is a glycoprotein comprised of two subunits, α and β, contains 30 % of carbohydrates, and has a molecular weight of about 36,700 daltons. The α-subunit is comprised of 92 amino acid residues, having the same structure as the α-subunit of luteinizing hormone and thyroid stimulating hormone. The β-subunit is comprised of 145 amino acid residues (structurally characteristic of hCG) (Lapthorn et al., Nature. 1994. Vol 369:455~461).

[0005] In general, the hCG is found to exist in various forms in the human body fluid. There is known intact-hCG (I-hCG), as well as modified hCGs including nicked hCG(N-hCG), hyperglycosylated hCG, free β-hCG and free β-core fragment. These various forms of hCGs are produced in the event of pregnancy, pituitary gland, trophoblast disease and choriocarcinoma. In the body fluid of a woman with a normal pregnancy, intact hCG comprises about 90 % of the total hCGs present and various modified hCGs are detected in an amount of about 10 % or less.

[0006] On the other hand, ectopic pregnancy is characterized in that almost entire hCGs are present in the form of intact hCG and there is very little amount of modified hCGs in contrast to normal pregnancy where certain ratios of nicked hCG and free β-hCG are detected. In addition, the total amount of hCG secretion and the range of increase are very small as compared with those in the normal pregnancy. Thus, the amount of hCG secretion reaches about 1/5 of that for the normal pregnancy at the 6^{th} week of pregnancy and decreases to about 1/50 of that for the normal pregnancy at the 8^{th} week of pregnancy, i.e., about 15 IU/ml.

[0007] U.S. Patent No. 5,786,220 discloses a process for the preparation of a one-step diagnostic reagent system that distinguishes between normal pregnancy and abnormal pregnancy. In this patent, it is disclosed that normal pregnancy, spontaneous abortion, ectopic pregnancy, cancers, etc. can be diagnosed by simultaneously determining the concentration of progesterone and the hCG level in the body fluid of a human female. Specifically, a method of diagnosis

is disclosed wherein a progesterone concentration of 25 ng/ml or lower in blood together with an hCG concentration of 25~2,500 mIU/ml is indicative of a spontaneous abortion or an ectopic pregnancy, and a progesterone concentration exceeding 25 ng/ml together with an hCG concentration exceeding 2,500 mIU/ml is indicative of a normal intrauterine pregnancy. Denil et al. (Fertility & Sterility. 1999. Vol. 72:1013~1017) disclosed that the free β-hCG level is 30~70 IU/ml in normal pregnancy, 1~70 IU/ml in abnormal intrauterine pregnancy, and 0.059~29 IU/ml in ectopic pregnancy, indicating that hCG degradation products are secreted to a much lower concentration in the ectopic pregnancy than in the normal pregnancy.

[0008] As described above, the hCG is the most important hormone in the diagnosis of pregnancy, and the hCG level in an early stage of pregnancy is utilized, in combination with ultrasound finings, for distinguishing between a pregnancy, an abortion, and an ectopic pregnancy. In the diagnosis of pregnancy, the hCG can be used for confirming a pregnancy at an early stage. A low level of hCG together with doubling time, which refers to the doubling of hCG in blood by every 1.4-2 days during the 5^{th} to the 9^{th} weeks from conception, indicates an abortion or an ectopic pregnancy. An hCG level higher than the normal value is interpreted as being indicative of multiple pregnancy or hydatidiform mole. The ectopic pregnancy is also diagnosed via ultrasonography, diagnostic laparoscopy and the like in addition to the measurement of serum and urine hCG level in a pregnant female. Particularly, the ultrasonography is conducted for diagnosis in combination with the hCG level measurement, wherein the presence of gestational sac In the ultrasound findings together with an hCG level below 1,000 mIU/mℓ indicates a sparse viability of the pregnancy. If the hCG level does not rise quantitatively by at least 65% per 48 hour, the prognosis of the pregnancy is determined to be very poor.

[0009] Such diagnostic methods for the ectopic pregnancy as the known methods of measuring the doubling time of the hCG level and the method of measuring the free β-hCG level in the body fluid of a pregnant female have the disadvantage in that repeated blood sampling is needed. The method for pregnancy diagnosis disclosed in U.S. Patent No. 5,786,220 is based on diagnostic principles different than those of the present invention in that the measurement of progesterone concentration should be conducted in parallel with the measurement of the hCG level. There is also another problem in that even though the distinction between a normal pregnancy and an abnormal pregnancy can be made, an effective early distinction between an ordinary spontaneous abortion and an ectopic pregnancy which are very hazardous events for a pregnant female is difficult to be achieved.

[0010] Cole et al. (The Journal of Clinical Endocrinology and Metabilism, 1994, Vol. 78, No. 2, pp. 497-499) disclose a method for diagnosis of ectopic pregnancy which comprises detection of I-hCG and ß-hCG core fragment (modified hCG) in two separate ELISA immunoassays using three different antibodies: anti-α-hCG, anti- ß-hCG and anti-modified hCG antibody. This method is complicated as it requires two separate determinations.

[0011] Accordingly, the present inventors have conducted extensive studies on the process for the preparation of a one-step simultaneous diagnosis kit by which the diagnosis of a normal pregnancy and an ectopic pregnancy can be achieved simultaneously in an early pregnancy diagnosis. As a result, the present inventors have now provided a one-step simultaneous diagnosis kit by which a normal pregnancy can be determined by detecting an hCG level of 25mIU/ml, and an ectopic pregnancy can be determined through comparison between the concentrations of I-hCG and modified hCGs.

Disclosure of Invention

[0012] An object of the present invention is to provide a diagnostic device for a one-step simultaneous diagnosis of normal pregnancy and ectopic pregnancy at an early stage of pregnancy.

[0013] The present invention as described in claims 1 and 2 is characterized in that an anti-I-hCG monoclonal antibody is used in combination with an anti-α-hCG monoclonal antibody and an anti-modified hCG monoclonal antibody is used in combination with an anti-β-hCG monoclonal antibody in order to respectively detect the I-hCG and modified hCGs present in the body fluid (blood, urine, saliva, etc.) of a pregnant female.

Brief Description of Drawings

[0014]

Fig. 1a shows a schematic view of a simultaneous diagnosis device for normal pregnancy and ectopic pregnancy according to the present invention. Reference numerals 1 and 4 designate reading windows of the diagnosis kit, and reference numerals 2 and 5 designate index (C) indicating the completion of the test. Reference numeral 3 designates an ectopic pregnancy line (hereinafter, referred to as "EP"), and reference numeral 6 designates a normal pregnancy line (hereinafter, referred to as "NP"). Reference numeral 7 designates a specimen receiving aperture to which a body fluid of a pregnant female is applied.

Fig. 1b schematically shows the result lines (NP, EP) and the completion line (C) on the antibody-immobilized membrane used for the inner strip in Fig. 1a. Reference numeral 11 designates a nitrocellulose membrane. Reference

numerals 8 and 12 designate completion lines where a rabbit anti-mouse immunoglobulin polyclonal antibody has been immobilized. Reference numeral 9 designates a test result line where an anti-β-hCG monoclonal antibody has been immobilized, and reference numeral 13 designates a test result line where an anti-α-hCG monoclonal antibody has been immobilized. Reference numeral 10 designates a pad of colored particulates having an anti-modified hCG monoclonal antibody bound thereon, and reference numeral 14 designates a pad of colored particulates having an anti-I-hCG monoclonal antibody bound thereon.

Fig. 2a shows another schematic view of a simultaneous diagnosis device for normal pregnancy and ectopic pregnancy according to the present invention. Reference numeral 15 designates an index (C) indicating the completion of the test. Reference numeral 16 designates an ectopic pregnancy line (EP) and reference numeral 14 designates a normal pregnancy line (NP). Reference numeral 18 designates a reading window of the diagnostic device, and reference numeral 19 designates a specimen receiving aperture to which a body fluid specimen of a pregnant female is applied.

Fig. 2b schematically shows the result lines (NP, EP) and the completion line (C) on the antibody-immobilized membrane used for the inner strip in Fig. 2a. Reference numeral 20 designates a nitrocellulose membrane. Reference numeral 21 designates a completion line where a rabbit anti-mouse immunoglobulin polyclonal antibody has been immobilized. Reference numeral 22 designates an ectopic pregnancy (EP) line where an anti-α-hCG monoclonal antibody has been immobilized, and reference numeral 23 designates a normal pregnancy (NP) line where an anti-β-hCG monoclonal antibody has been immobilized. Reference numeral 24 designates a pad of mixed colored particulates having an anti-modified hCG monoclonal antibody and an anti-I-hCG monoclonal antibody bound thereon.

Fig. 3 shows a construction of the inner strip in the simultaneous diagnosis device for normal pregnancy and ectopic pregnancy of the present invention. Reference numeral 25 designates a specimen absorbing pad, reference numeral 26 designates an antibody-immobilized nitrocellulose membrane, reference numeral 27 designates a colored particulate pad, and reference numeral 28 designates a specimen receiving pad, wherein these pads are combined sequentially in a partially overlapping manner.

Best Mode for Carrying Out the Invention

[0015] The present invention is based on an immunological assay method called sandwich assay. In such an assay, the selection of the antibodies used in the assay is very important because the antibodies dictate the sensitivity and the specificity for a specimen to be assayed. In a sandwich assay, two kinds of monoclonal antibodies are primarily used, where the binding sites for the antibodies should be present on different regions of the relevant antigen in order to maintain the assay sensitivity. For example, in order to examine the hCG which is secreted into the body fluid (blood, urine, saliva, etc.) of a pregnant female using the sandwich assay, two kinds of monoclonal antibodies are needed and the binding sites on the antigen (hCG) for the two antibodies should be located distinctively apart. If the binding sites on the antigen (hCG) for the two antibodies used are located in similar or close regions, steric hindrance may lead to a decrease in the assay sensitivity. The present invention utilizes an immuno-chromatographic method based on the sandwich assay among immunological assay methods. Particularly, a previously characterized monoclonal antibody is bound to colored fine particles (colored particulates) by covalent or noncovalent bonding, the resulting particulates being used as the mobile phase. A second, previously characterized monoclonal antibody is dispensed into and immobilized onto a nitrocellulose membrane to be used as the solid phase. The specimen to be assayed is mixed with the mobile phase and run, by the capillary action, through the membrane as the solid phase. Here, if a relevant antigen is contained in the specimen, the mobile phase and the solid phase are conjugated via the antigen, resulting in an observable line of the colored particulates appearing on the solid phase. From the appearance or absence of such an observable line, the presence or absence of the relevant antigen in the specimen is determined visually. Monoclonal antibodies to be used in the present invention may be purchased as commercially available monoclonal antibody or prepared according to known cell fusion protocols. The antibodies used in the Examples were prepared according to a known process for preparing monoclonal antibody, where antibodies reactive to I-hCG and antibodies reactive to modified hCG were selected. For the anti-α-hCG antibody and anti-β-hCG antibody, monoclonal antibodies were selected and used, which showed a good maintenance of sensitivity when examined using standard hCG.

[0016] As described above, the hCG is present in various forms in the urine of a female with normal pregnancy or ectopic pregnancy at an early stage of pregnancy. In particular, most of the hCG found in the case of the ectopic pregnancy is in the intact form (I-hCG), with very little amount of modified hCG present. In the case of the normal pregnancy, I-hCG comprises about 90% of the total hCGs present, and various modified hCGs comprise about 10%. Utilizing the above difference, the present invention allows the confirmation of a pregnancy simultaneously with the distinction between normal pregnancy and ectopic pregnancy by separately detecting and visually comparing I-hCG and modified hCGs. The construction of the diagnostic device of the present invention is completed by separately providing a diagnosis kit with an ectopic pregnancy (EP) region where a colored line appears when the I-hCG is present

in the specimen and with a normal pregnancy (NP) region where a colored line appears when modified-hCGs are present in the specimen. In the case of an ectopic pregnancy, most of the hCG is present in the form of the I-hCG. Consequently, a colored line will appear only in the EP region due to the reaction of I-hCG, or, even if an NP line appears, a much more intensive EP line will appear. In a normal pregnancy, about 10 % of modified hCGs are present in the body fluid in addition to the I-hCG. Accordingly, in the diagnostic device of the present invention, the concentration of colored particulates having an antibody reactive to modified hCGs bound thereon is increased 2~10-fold, or the concentration of the antibody to be bound is correspondingly increased so as to enhance the reaction sensitivity with the modified hCGs. It would render the intensities of the colored lines appearing in the two regions (EP, NP) to be similar to assist in diagnosing a normal pregnancy.

[0017] Hereinafter, the construction of the pregnancy diagnosis device according to the present invention will now be described in more detail.

[0018] Monoclonal antibody against I-hCG, which is overexpressed in an ectopic pregnancy, is prepared according to a well-known method and then bound to colored particulates. The resulting particulates are incorporated into a glass fiber pad and dried to produce a colored particulate pad. On the other hand, monoclonal antibody against modified hCGs is prepared according to a well-known method and then bound to colored particulates. The resulting particulates are incorporated into a glass fiber pad and dried to produce a colored particulate pad. Various colored particulates can be used in the present invention including polystyrene particles, colloidal gold, and the like. Among such particles, the colloidal gold is preferable and the colloidal gold of 20 to 60 nm in size is more preferable.

[0019] In order to maintain the sensitivity of the diagnostic device, monoclonal antibodies that bind to binding sites on the antigen (hCG) which is different than the binding sites for the antibodies bound to the colored particulates were used for the solid phase membrane. The monoclonal antibodies used for this purpose are an anti-β-hCG monoclonal antibody and an anti-α-hCG monoclonal antibody. The two antibodies are immobilized on separate nitrocellulose membranes by dispensing the antibody into the membrane to respectively form a straight line (Fig. 1), or immobilized on one membrane by dispensing the antibodies into the membranes to form two crossing lines or two separate lines (Fig. 2). At an end of the membrane downstream of the monoclonal antibody-bound zone, an anti-mouse immunoglobulin polyclonal antibody is dispensed into the membrane so that a test completion line will appear.

[0020] The antibody- immobilized membrane prepared as described above may be attached to a polyester support having an adhesive applied thereon, and then the monoclonal antibody-colored particulate pad is applied thereover in the longitudinal direction. Subsequently, a specimen receiving pad on which the body fluid of a pregnant female is to be directly dropped, and a specimen absorbing pad for absorbing excess body fluid are applied in the longitudinal direction in a manner that the pads overlaps with each other, providing the inner strip of the simultaneous diagnosis kit for normal pregnancy and ectopic pregnancy (Fig. 3).

[0021] Methods for an early diagnosis of normal pregnancy and ectopic pregnancy using a simultaneous diagnosis device prepared as describe above will now be illustrated.

[0022] When the body fluid (blood, urine, saliva, etc.) specimen of a pregnant female is applied to the specimen receiving pad, the specimen is absorbed and transported by the capillary action, and then reacts with the monoclonal antibody-bound colored particulates which are incorporated in the colored particulate pad. In the case of normal pregnancy, I-hCG and modified hCGs present in the specimen will respectively bind to monoclonal antibodies bound on the colored particulates and move along the nitrocellulose membrane phase. In those regions where two kinds of monoclonal antibodies are immobilized on the membrane, the antigen-antibody complexes will respectively bind in a sandwich form, resulting in two result lines (NP, EP) formed by colored particulates appearing on the membrane with similar intensities.

[0023] In ectopic pregnancy, I-hCG comprises most of the hCGs present in the body fluid. Accordingly, the complex of the I-hCG-bound monoclonal antibody and colored particulates will move along the membrane and bind to the anti-α-hCG monoclonal antibody to produce a strong EP band. In contrast, colored particulates having monoclonal antibody against modified hCG bound thereon will not react with I-hCG in the specimen, and consequently will move on without binding to the anti-β-hCG monoclonal antibody immobilized on the membrane. The particulates may also react with the extremely small amount of modified hCGs present in the specimen, producing an NP band with a very weak intensity. Thus, in the case of ectopic pregnancy, either one EP band only or two bands with different line intensities (EP band stronger than NP band) will appear.

[0024] If the tested female is not pregnant, the monoclonal antibody-colored particulate complex will move on without binding to the anti-hCG monoclonal antibody immobilized on the result lines (NP, EP) in the membrane because hCG is not present in the specimen. Consequently, the colored particulate band will appear only on the test completion line (C) where the anti-mouse immunoglobulin polyclonal antibody has been immobilized.

[0025] Examples depicting the shapes and structures of the simultaneous diagnosis device of the present invention for normal pregnancy and ectopic pregnancy are shown in Figs. 1a to 2b.

[0026] The same results may be obtained if the antibodies bound to the colored particulate pad and the antibodies bound to the membrane are interchanged. An anti-α-hCG monoclonal antibody and an anti-β-hCG monoclonal antibody are respectively bound to colored particulates, and an anti-I-hCG monoclonal antibody, an anti-modified hCG monoclonal

antibody and an anti-mouse immunoglobulin polyclonal antibody are immobilized on nitrocellulose membranes as described above to provide a diagnosis kit. When a body fluid from a pregnant female is applied to the specimen receiving pad, 1) in normal pregnancy, I-hCG and other modified hCGs present in the specimen bind to the anti-α-hCG monoclonal antibody and the anti-β-hCG monoclonal antibody respectively, bound to colored particulates and move along the nitrocellulose membrane, and then respectively bind in a sandwich manner to the anti-I-hCG monoclonal antibody and the anti-modified hCG monoclonal antibody immobilized on the membrane, resulting in two result lines (NP, EP) appearing on the membrane with similar intensity; 2) in ectopic pregnancy, I-hCG present in the body fluid binds to the anti-α-hCG monoclonal antibody bound to colored particulates and moves along the membrane, and then binds to the anti-I-hCG monoclonal antibody on the membrane, giving a strong EP band only, or the extremely small amount of modified hCG present in the specimen produces an NP band with a very weak intensity; 3) if the tested female is not pregnant, the monoclonal antibody-colored particulate complex will move on without binding to the anti-hCG monoclonal antibody immobilized on the result lines (NP, EP) in the membrane because hCG is not present in the specimen. Consequently, the colored particulate band will appear only on the test completion line (C) where the anti-mouse immunoglobulin polyclonal antibody has been immobilized.

[0027] The present invention will now be described in more detail with reference to the following examples. It should be appreciated by a person skilled in the art that these examples are presented for the purpose of illustration only and that the examples should in no way be interpreted to limit the scope of the present invention.

Example 1: Preparation and purification of an anti-I-hCG monoclonal antibody and an anti-α-hCG monoclonal antibody

A. Immunization and cell fusion

[0028] A known cell-fusion procedure (Galfre, G. et al. 1981, Methods Enzymol. 73:3-46) was used to prepare a monoclonal antibody against I-hCG. First, 20μg/100μℓ of I-hCG (Zymed, USA) was fully emulsified with 100μℓ of Freund's complete adjuvant and injected into a Balb/C mouse (8 weeks) i.p. After 3 weeks, a second i.p. injection was conducted under the same protocol as the first injection except that 100μℓ of Freund's incomplete adjuvant was used for the emulsification. After 1 week, blood samples were collected from the mouse and the antibody formation was determined by an ELISA , after which 20μg of hCG was injected i.v. in the tail. Three days later, spleen cells were recovered from the mouse and cell fusion was performed using pre-cultured Sp2/O cells and PEG. The fused cells were cultured in a 96-well plate with the addition of HAT medium. Then, cells secreting antibodies reactive to I-hCG and cells secreting antibodies reactive to α-hCG were selected and subjected to large-scale culture.

B. Purification of monoclonal antibody

[0029] Large-scale cultures were centrifuged to remove precipitates. The supernatants were pulled and loaded onto a protein A-sepharose(FF) column, rinsed with a phosphate buffer and eluted with 0.1M glycine buffer. The eluate was dialysed against a phosphate buffer to adjust the concentration and used for the preparation of diagnosis kits.

Example 2: Preparation and purification of an anti-modified hCG monoclonal antibody and an anti-β-hCG monoclonal antibody

[0030] Spleen cells were obtained from a mouse which had been immunized three times with a β-hCG antigen purchased from Zymed according to the same procedure as in Example 1, A. The cells were admixed with Sp2/O cells and cell fusion was conducted using PEG. The fused cells were cultured in a 96-well plate with the addition of HAT medium. Then, cells secreting antibodies reactive to β-hCG and cells secreting antibodies reactive to modified hCGs without reactivity to intact hCG were selected and subjected to large-scale culture. Subsequently, monoclonal antibodies were purified from large-scale cultures according to the same purification procedures as in Example 1, B, adjusted the concentration after dialyzing against a phosphate buffer, and then used for the preparation of diagnosis kits.

Example 3: Preparation of colloidal gold (colored particulates)

[0031] Colloidal gold of 20-60 nm in size was used as colored particulates. To prepare the colloidal gold, 220 ml of double distilled water was put into a 500 ml round-bottomed flask. The flask was then placed over a hot plate (Corning, USA) and a reflux apparatus (Pyrex, USA) was equipped to prevent the evaporation of water. The hot plate was turned on to heat the flask to 100 °C with suspension. When the temperature of the distilled water exceeds 100°C, 1.0 ml of 2% gold chloride (Sigma, USA) was added with intimate mixing followed by 2.0ml of 1% sodium citrate (Sigma, USA). Heating was continued for further 30 minutes to produce colloidal gold. The colloidal gold thus produced was filtered on a 0.45μm filter paper to remove impurities and aggregates, and then used for the preparation of diagnostic device.

Example 4: Preparation of colored particulate pads in which colored particulates having a monoclonal antibody bound thereto are incorporated

[0032] To prepare colored particulates having a monoclonal antibody bound thereto, 50 ml aliquots of the colored particulates prepared in Example 3 were placed individually in beakers. Monoclonal antibodies prepared in Examples 1 and 2 were respectively added into the flasks in an amount of 1 ~ 15μg per 1 ml of colored particulates with stirring, and reacted for 2 to 30 min. After completion of the reaction, a blocking solution containing 1~10% bovine serum albumin was added into each beaker to a final albumin concentration of 0.1~1% and reacted for 2 to 30 min.

[0033] The reaction mixture was put into 50ml centrifuge tubes (Corning, USA) and centrifuged in an ultracentrfuge (Backman, USA) at 10,000 rpm for 15 min. After centrifuge, the supernatants were discarded, and the precipitates were removed and resuspended in a stabilizing buffer (0.5~2% bovine serum albumin, 1~5% sucrose, 50~100mM Tris·HCl buffer (pH 7.5~9.0)). The resulting suspensions were adjusted for an adequate concentration, and colored particulate pads were soaked in the suspensions and then dried. Colored particulates having the anti-modified hCG monoclonal antibody obtained in Example 2 bound thereto were added to a concentration as high as 2 to 10 times the concentration of colored particulates having the anti-I-hCG monoclonal antibody obtained in Example 1 bound thereto. Alternatively, a 2 to 10 fold higher concentration of the anti-modified hCG monoclonal antibody was used for the binding reaction, such that modified hCGs, which are contained in the test specimen in a relatively small amount (10%), can be clearly detected.

[0034] When the anti-β-hCG monoclonal antibody obtained in Example 2 and the anti-α-hCG monoclonal antibody obtained in Example 1 were formulated into colored particulate pads, the concentration of the anti-modified hCG monoclonal antibody which was bound to the membrane was adjusted to be 2 to 10 times higher than the concentration of the anti-I-hCG monoclonal antibody so that modified hCGs can be clearly detected.

Example 5: Preparation of antibody-immobilized membranes (Readout panel)

[0035] To make result lines reacting in response to normal pregnancy and ectopic pregnancy within zones where an antibody is immobilized, monoclonal antibody recognizing and binding α-hCG and monoclonal antibody recognizing and binding β-hCG were dropped onto a nitrocellulose membrane to respectively form straight lines. The antibodies were prepared at a concentration of 1 to 4.0 mg/ml and dropped and immobilized on the membrane to a final concentration of 0.1ug to 5ug/cm, where a 5~50mM phosphate buffer containing 0.1 to 2 % sucrose was used as the immobilization solution. Downstream to the monoclonal antibody-dropped zone, reaction completion line (C in Fig. 1a and Fig. 2b) was provided using a rabbit anti-mouse immunoglobulin (IgG) polyclonal antibody at a concentration of 0.5~2 mg/ml, wherein a 5~50mM phosphate buffer containing 0.1 to 2 % sucrose was used as the immobilization solution. Membranes where the antibody immobilization had been completed were dried at room temperature for 2 hours. The antibody to be immobilized and the drop pattern can be varied according to the shape of the result lines to be displayed. The diagnosis kit shown in Fig. 1 was prepared by dropping and immobilizing an anti-α-hCG monoclonal antibody and an anti-β-hCG monoclonal antibody on different membranes. In the diagnosis kit shown in Fig. 2, the two antibodies were dropped and immobilized on one membrane in the form of two straight lines spaced apart in order to make the result lines appear on one membrane. Particularly for a diagnosis kit as shown in Fig. 1, the selection of the antibody to be immobilized on the membrane and the antibody to be bound to the colored particulates is very important for obtaining the desired results. Thus, when a membrane having an anti-α-hCG monoclonal antibody immobilized thereon is used, a colored particulate pad having an anti-I-hCG monoclonal antibody bound thereto is desirably used. When a membrane having an anti-β-hCG monoclonal antibody immobilized thereon is used, a colored particulate pad having an anti-modified hCG monoclonal antibody bound thereto is desirably used so that modified hCG can be detected. When a membrane having an anti-modified hCG monoclonal antibody immobilized thereon is used, a colored particulate pad having an anti-β-hCG monoclonal antibody bound thereto is used. If a membrane having an anti-I-hCG monoclonal antibody immobilized thereon is used, a colored particulate pad having an anti-α-hCG monoclonal antibody bound thereto is desirably used.

Example 6: Specimen receiving pad and specimen absorbing pad

[0036] Glass fiber (Millipore, USA) or cellulose paper (Whatman, England) was used in the specimen receiving pad and cellulose paper (Whatman, England) was used in the specimen absorbing pad.

Example 7: Preparation of a strip for simultaneous diagnosis of pregnancy and ectopic pregnancy

[0037] As depicted in Fig. 3, the antibody-immobilized membrane prepared in Example 5 was attached to an adhesive polyester support, and the antibody-bound colored particulate pad prepared in Example 4 was applied thereover with an overlap of 1~3mm. The specimen receiving pad was then applied with an overlap of 1~10mm in the longitudinal

direction followed by the specimen absorbing pad with an overlap of 1~5mm to give a simultaneous diagnosis strip. The resulting simultaneous diagnosis strip was assembled into a plastic housing such as those shown in Figs. 1a and 2a to produce a simultaneous diagnosis kit for normal pregnancy and ectopic pregnancy.

Experimental Example 1: Evaluation of the simultaneous diagnosis kit using an hCG standard solution.

[0038] To evaluate the specificity of the diagnosis kit prepared according to the above Examples, an hCG standard solution was prepared from I-hCG commercially available from Zymed (Cat. No. 14-1401) and the urine of a pregnant female at her early stage of pregnancy who had been determined to have a normal pregnancy on the basis of ultrasound findings. Colored particulate pads for the kit to be evaluated were prepared by binding a monoclonal antibody against I-hCG and a monoclonal antibody against modified hCGs respectively to colored particulates as in Example 4. Antibody bound membranes were prepared by dropping an anti-α-hCG monoclonal antibody and an anti-β-hCG monoclonal antibody respectively on nitrocellulose membranes as in Example 5. The resulting two kinds of antibody-fixed membranes and two kinds of colored particulate pads were assembled to provide every possible combination of membrane and pad. Then, specimen receiving pads and specimen absorbing pads were respectively attached to the assemblies to produce simultaneous diagnosis strips.

[0039] As standard solutions, negative and positive standards were prepared and used in the tests.

Negative standard: 50mM phosphate buffer (pH 7.2)
Positive standard 1: I-hCG 50mIU/ml phosphate buffer (pH 7.2)
Positive standard 2: Urine from a female of an early stage pregnancy wherein ultrasound findings confirmed the normal intrauterine gestational sac and the heart beat of the fetus had been detected.

[0040] The above standard solutions were applied to the specimen receiving zones at an amount of about 500$\mu\ell$ and observed for 3 minutes. The appearance or absence of a result line indicated "positive" and "negative" for the result.

[0041] The test results are listed in Table 1 below.

Table 1. Comparison of recognition properties for the monoclonal antibodies used for the simultaneous diagnosis kits (1)

Standard	Antibody bound on membrane	Antibody bound to colored particulates	
		anti-modified hCG monoclonal antibody	anti-I-hCG monoclonal antibody
Negative standard	anti-β-hCG antibody	-	-
	anti-α-hCG antibody	-	-
Positive standard 1	anti-β-hCG antibody	+	+
	anti-α-hCG antibody	+	++
Positive standard 2	anti-β-hCG antibody	+++	+
	anti-α-hCG antibody	+	+++
Notes: - (no reaction), + (very little reaction), ++(significant reaction), +++ (strong reaction)			

[0042] As can be seen from Table 1, in the case of the negative standard which does not contain hCG, the result is determined to be "negative" because there appear no lines within those zones on the membrane where antibody 1 and 2 are immobilized. For the positive standard 1 containing I-hCG, colored particulates having an anti-I-hCG monoclonal antibody bound thereto and the zone where an anti-α-hCG monoclonal antibody is immobilized showed a strong interaction and kits with other monoclonal antibodies showed weak interactions. For the positive standard 2 utilizing urine from a female with a normal pregnancy, colored particulates having an anti-I-hCG monoclonal antibody bound thereto and the zone where an anti-α-hCG monoclonal antibody is immobilized showed a strong interaction. Colored particulates having an anti-modified hCG monoclonal antibody bound thereto and the zone where an anti-β-hCG monoclonal antibody is immobilized also showed a strong interaction. From these results, it could be recognized that an anti-I-hCG monoclonal antibody in combination with an anti-α-hCG monoclonal antibody can detect I-hCG, while the detection of modified hCGs can be achieved by using an anti-modified hCG monoclonal antibody and an anti-β-hCG monoclonal antibody.

[0043] Similar results were obtained when the antibodies bound to colored particulates and the membrane were interchanged, clearly indicating the importance of the combination of the antibodies used in the assay. Table 2 shows

the results from a test with a standard hCG solution, wherein an anti-α-hCG monoclonal antibody and an anti-β-hCG monoclonal antibody are bound to colored particulates and an anti-I-hCG monoclonal antibody and an anti-modified hCG monoclonal antibody were immobilized on the membrane to prepare a simultaneous diagnosis kit.

Table 2. Comparison of recognition properties for the monoclonal antibodies used for the simultaneous diagnosis kits (2)

Standard	Antibody bound on membrane	Antibody bound to colored particulates	
		anti-β-hCG monoclonal antibody	anti-α-hCG monoclonal antibody
Negative standard	anti-modified hCG antibody	-	-
	anti-I-hCG antibody	-	-
Positive standard 1	anti-modified hCG antibody	+	+
	anti-I-hCG antibody	+	++
Positive standard 2	anti-modified hCG antibody	+++	+
	anti-I-hCG antibody	+	+++
Notes: - (no reaction), + (very little reaction), ++(significant reaction), +++ (strong reaction)			

[0044] As seen in the above experimental example, normal pregnancy and ectopic pregnancy could be determined with naked eyes because the location and intensity of the appearing lines varied depending on the form of the hCG contained in the specimen.

[0045] Experimental Example 2: Assay on the urine of a pregnant female using a kit for the simultaneous diagnosis of normal pregnancy and ectopic pregnancy

[0046] Table 3 shows the results from a test where urine samples obtained from a non-pregnant female, a female with normal pregnancy, and a female with ectopic pregnancy were applied respectively to simultaneous diagnosis kits of the present invention for assaying modified-hCGs and I-hCG which are specifically found in nomial pregnancy and ectopic pregnancy. The urine sample for normal pregnancy was obtained from a pregnant female for whom ultrasound finings confirmed intrauterine gestational sac and the heart beat of the fetus had been detected. The urine sample for ectopic pregnancy was obtained from a female for whom the ectopic pregnancy findings were confirmed through a surgical operation. The diagnosis kits used in this example were in the form shown in Fig. 1a.

[0047] As is seen from Table 3, no lines appeared at the result lines for NP and EP in the case of the urine sample from a non-pregnant female. In the case of the urine sample from a female with normal pregnancy, the result lines for NP and EP appeared with similar intensity. In particular, for the urine sample from a female with ectopic pregnancy, a much stronger line appeared at the result line for EP than that for NP, which means that the readout allows a sharp distinction between normal pregnancy and ectopic pregnancy.

Table 3. Determination of clinical accuracy of the simultaneous diagnosis kit

Specimen / Type		1	2	3	4	5	6	7	8	9	10
Urine (non-pregnant)	NP	-	-	-	-	-	-	-	-	-	-
	EP	-	-	-	-	-	-	-	-	-	-
Urine (normal pregnancy)	NP	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	EP	+++	+++	+++	+++	++	+++	+++	++	+++	+++
Urine (ectopic pregnancy)	NP	+	-	+	+	-	-	+	-	-	+
	EP	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Notes: - (no reaction), + (very little reaction), +++ (strong reaction)

Industrial Applicability

[0048] The present invention provides one-step pregnancy diagnosis devices that can simultaneously detect normal pregnancy and ectopic pregnancy and methods for the preparation of such devices. Since the body fluid of a pregnant female contains different forms of hCG depending on the form of the pregnancy, the present invention allows a rapid and accurate detection of normal pregnancy and ectopic pregnancy at an early pregnancy stage, by immunologically detecting the forms of hCG present in the body fluid. In the present invention, an anti-I-hCG monoclonal antibody is combined with an anti-α-hCG monoclonal antibody and an anti-modified hCG monoclonal antibody is combined with an anti-β-hCG monoclonal antibody in order to individually detect I-hCG and modified hCGs present in the body fluid of a pregnant female. Consequently, the present invention will be useful in reducing the maternity mortality by finding ectopic pregnancy at an early stage.

Claims

1. A pregnancy diagnosis device comprising:

a) a specimen receiving pad and a specimen absorbing pad;
b) a particulate pad comprising colored particulates bound to anti-α-hCG and anti-β-hCG monoclonal antibodies for detecting hCGs (human chorionic gonadotropins) in a specimen, said colored particulates formed from one or more selected from selenium, gold and polystyrene;
c) a membrane having a plurality of lines including:

(i) an ectopic pregnancy line defined by the presence of an anti-intact-hCG monoclonal antibody;
(ii) a normal pregnancy line defined by presence of an anti-modified hCG monoclonal antibody which is reactive to modified hCGs but not reactive to intact hCG; and
(iii) a test completion line defined by presence of an anti-mouse immunoglobulin polyclonal antibody,

wherein a line becomes visible when analyte bound to colored particulates is captured by a cognate antibody and wherein an ectopic pregnancy is indicated by a stronger ectopic pregnancy line relative to the normal pregnancy line, and wherein said modified hCGs are selected from nicked hCG, hyperglycosylated hCQ free ß-hCG and free ß-core fragment.

2. A pregnancy diagnosis device comprising:

a) a specimen receiving pad and a specimen absorbing pad;
b) a particulate pad comprising colored particulates bound to anti-intact-hCG and anti-modified-hCG monoclonal antibodies for detecting hCGs in a specimen, said colored particulates formed from one or more selected from selenium, gold and polystyrene, and said anti-modified-hCG monoclonal antibodies being reactive to modified hCGs but not reactive to intact hCG;
c) a membrane having a plurality of lines including:

(i) an ectopic pregnancy line defined by the presence of an anti-α-hCG monoclonal antibody;
(ii) a normal pregnancy line defined by presence of an anti-ß-hCG monoclonal antibody; and
(iii) a test completion line defined by presence of an anti-mouse immunoglobulin polyclonal antibody,

wherein a line becomes visible when analyte bound to colored particulates is captured by a cognate antibody and wherein an ectopic pregnancy is indicated by a stronger ectopic pregnancy line relative to the normal pregnancy line, wherein said modified hCGs are selected from nicked hCG, hyperglycosylated hCG, free ß-hCG and free ß-core fragment.

3. The pregnancy diagnosis device according to claim 1 or 2 wherein the anti-intact-hCG monoclonal antibody and the anti-modified hCG monoclonal antibody are used at a concentration ratio of 1:2-10.

4. The pregnancy diagnosis device according to any one of claims 1 to 3 wherein
when a specimen from a non-pregnant female is tested, only the test completion line is visually observable;
when a specimen from a female with a normal pregnancy is tested, the test completion line, the ectopic pregnancy line and the normal pregnancy line are visually observable with equal intensities; and when a specimen from a female with an ectopic pregnancy is tested, only the test completion line and the ectopic pregnancy line are visually observable, or alternatively the test completion line, the ectopic pregnancy line, and the normal pregnancy line are turned visually observable with the intensity of the normal pregnancy line being weaker than the test completion and ectopic pregnancy lines.

5. The pregnancy diagnosis device according to any one of claims 1 to 4 wherein the anti-α-hCG monoclonal antibody and the anti-β-hCG monoclonal antibody has been dissolved in an immobilization buffer containing 0.1%-2% sucrose and 5-50 mM phosphate buffer and then immobilized onto the membrane to 0.1-5 μg/cm.

6. The pregnancy diagnosis device according to any one of claims 1 to 5 wherein the specimen is selected from urine, blood and saliva.

Patentansprüche

1. Schwangerschafts-Diagnosevorrichtung, umfassend

a) ein probenaufnehmendes Pad und ein probenabsorbierendes Pad,
b) ein partikuläres Pad umfassend gefärbte Teilchen, die an Anti-α-hCG und Anti-β-hCG monoklonalen Antikörpern zum Nachweisen von hCGs (humanen Choriongonadotropinen) in einer Probe gebunden sind, wobei die gefärbten Teilchen aus einem oder mehreren ausgewählt aus Selen, Gold und Polystyrol gebildet sind,
c) eine Membran mit einer Mehrzahl von Linien einschließlich:

(i) einer ektopischen Schwangerschaftslinie, die durch die Anwesenheit eines Anti-intakten-hCG monoklonalen Antikörpers definiert ist;
(ii) einer normalen Schwangerschaftslinie, die durch die Anwesenheit eines Anti-modifizierten-hCG monoklonalen Antikörpers definiert ist, der mit modifizierten hCGs reaktiv ist, aber mit intaktem hCG nicht reaktiv ist; und
(iii) einer Testfertigstellungslinie, die durch die Anwesenheit eines Anti-Maus-Immunglobulin polyklonalen Antikörpers definiert ist,

wobei eine Linie sichtbar wird, wenn Analyt, der an gefärbten Teilchen gebunden ist, durch einen kognaten Antikörper eingefangen wird, und wobei eine ektopische Schwangerschaft durch eine stärkere ektopische Schwangerschafts-

linie relativ zu der normalen Schwangerschaftslinie angezeigt wird, und wobei die modifizierten hCGs ausgewählt sind aus nicked hCG, hyperglykosyliertem hCG, freiem β-hCG und freiem β-Core-Fragment.

2. Schwangerschafts-Diagnosevorrichtung, umfassend

a) ein probenaufnehmendes Pad und ein probenabsorbierendes Pad,
b) ein partikuläres Pad umfassend gefärbte Teilchen, die an Anti-intakten-hCG und Anti-modifizierten-hCG monoklonalen Antikörpern zum Nachweisen von hCGs in einer Probe gebunden sind, wobei die gefärbten Teilchen aus einem oder mehreren ausgewählt aus Selen, Gold und Polystyrol gebildet sind, und die Anti-modifizierten-hCG monoklonalen Antikörper mit modifizierten hCGs reaktiv sind, aber mit intaktem hCG nicht reaktiv sind,
c) eine Membran mit einer Mehrzahl von Linien einschließlich:

(i) einer ektopischen Schwangerschaftslinie, die durch die Anwesenheit eines Anti-α-hCG monoklonalen Antikörpers definiert ist;
(ii) einer normalen Schwangerschaftslinie, die durch die Anwesenheit eines Anti-β-hCG monoklonalen Antikörpers definiert ist; und
(iii) einer Testfertigstellungslinie, die durch die Anwesenheit eines Anti-Maus-Immunglobulin polyklonalen Antikörpers definiert ist,

wobei eine Linie sichtbar wird, wenn Analyt, der an gefärbten Teilchen gebunden ist, durch einen kognaten Antikörper eingefangen wird, und wobei eine ektopische Schwangerschaft durch eine stärkere ektopische Schwangerschaftslinie relativ zu der normalen Schwangerschaftslinie angezeigt wird, wobei die modifizierten hCGs ausgewählt sind aus nicked hCG, hyperglykosyliertem hCG, freiem β-hCG und freiem β-Core-Fragment.

3. Schwangerschafts-Diagnosevorrichtung nach Anspruch 1 oder 2, wobei der Anti-intakte-hCG monoklonale Antikörper und der Anti-modifizierte-hCG monoklonale Antikörper in einem Konzentrationsverhältnis von 1 : 2-10 verwendet werden.

4. Schwangerschafts-Diagnosevorrichtung nach irgendeinem der Ansprüche 1 bis 3, wobei
wenn eine Probe von einer nicht schwangeren Frau getestet wird, nur die Testfertigstellungslinie visuell beobachtbar ist;
wenn eine Probe von einer Frau mit einer normalen Schwangerschaft getestet wird, die Testfertigstellungslinie, die ektope Schwangerschaftslinie und die normale Schwangerschaftslinie mit gleichen Intensitäten visuell beobachtbar sind; und wenn eine Probe von einer Frau mit einer ektopischen Schwangerschaft getestet wird, nur die Testfertigstellungslinie und die ektope Schwangerschaftslinie visuell beobachtbar sind oder alternativ die Testfertigstellungslinie, die ektope Schwangerschaftslinie und die normale Schwangerschaftslinie ins visuell Beobachtbare umschlagen, wobei die Intensität der normalen Schwangerschaftslinie schwächer ist als die Testfertigstellungs- und die ektope Schwangerschaftslinie.

5. Schwangerschafts-Diagnosevorrichtung nach irgendeinem der Ansprüche 1 bis 4, wobei der Anti-α-hCG monoklonale Antikörper und der Anti-β-hCG monoklonale Antikörper in einem Immobilisierungspuffer gelöst worden sind, der 0,1% bis 2% Sucrose und 5 bis 50 mM Phosphatpuffer enthält, und dann auf der Membran mit 0,1 bis 5 μg / cm immobilisiert werden.

6. Schwangerschafts-Diagnosevorrichtung nach irgendeinem der Ansprüche 1 bis 5, wobei die Probe aus Urin, Blut und Speichel ausgewählt ist.

Revendications

1. Dispositif de diagnostic de grossesse, comprenant :

a) un tampon récepteur d'échantillon et un tampon d'absorption d'échantillon ;
b) un tampon comportant des particules, qui comprend des particules colorées liées à des anticorps monoclonaux anti-α-hCG et anti-β-hCG pour détecter les hCG (gonadotrophines chorioniques humaines) dans un échantillon, lesdites particules colorées étant formées à partir d'un ou plusieurs agents choisis entre le sélénium, l'or et le polystyrène ;

c) une membrane comportant une pluralité de lignes, comprenant :

(i) une ligne de détermination de grossesse extra-utérine, définie par la présence d'un anticorps monoclonal anti-hCG intacte ;
(ii) une ligne de détermination de grossesse normale, définie par la présence d'un anticorps monoclonal anti-hCG modifiée qui est réactif avec des hCG modifiées, mais qui n'est pas réactif avec la hCG intacte ; et
(iii) une ligne d'achèvement de test, définie par la présence d'un anticorps polyclonal anti-immunoglobuline de souris,

dans lequel une ligne devient visible lorsqu'un analyte lié à des particules colorées est capturé par un anticorps correspondant et dans lequel une grossesse extra-utérine est indiquée par une ligne de détermination de grossesse extra-utérine plus intense que la ligne de détermination de grossesse normale, et
dans lequel lesdites hCG modifiées sont choisies entre une hCG présentant une coupure, une hCG hyperglycosylée, la β-hCG libre et un fragment central β libre.

2. Dispositif de diagnostic de grossesse, comprenant :

a) un tampon récepteur d'échantillon et un tampon d'absorption d'échantillon ;
b) un tampon comportant des particules, qui comprend des particules colorées liées à des anticorps monoclonaux anti-hCG intacte et anti-hCG modifiée pour détecter les hCG dans un échantillon, lesdites particules colorées étant formées à partir d'un ou plusieurs agents choisis entre le sélénium, l'or et le polystyrène, et lesdits anticorps monoclonaux anti-hCG modifiée étant réactifs avec des hCG modifiées, mais n'étant pas réactif avec la hCG intacte ;
c) une membrane comportant une pluralité de lignes, comprenant :

(i) une ligne de détermination de grossesse extra-utérine, définie par la présence d'un anticorps monoclonal anti-α-hCG ;
(ii) une ligne de détermination de grossesse normale, définie par la présence d'un anticorps monoclonal anti-β-hCG ; et
(iii) une ligne d'achèvement de test, définie par la présence d'un anticorps polyclonal anti-immunoglobuline de souris,

dans lequel une ligne devient visible lorsqu'un analyte lié à des particules colorées est capturé par un anticorps correspondant et dans lequel une grossesse extra-utérine est indiquée par une ligne de détermination de grossesse extra-utérine plus intense que la ligne de détermination de grossesse normale,
lesdites hCG modifiées étant choisies entre une hCG présentant une coupure, une hCG hyperglycosylée, la β-hCG libre et un fragment central β libre.

3. Dispositif de diagnostic de grossesse suivant la revendication 1 ou 2, dans lequel l'anticorps monoclonal anti-hCG intacte et l'anticorps monoclonal anti-hCG modifiée sont utilisés en un rapport de concentration de 1:2-10.

4. Dispositif de diagnostic de grossesse suivant l'une quelconque des revendications 1 à 3, dans lequel
lorsqu'un échantillon provenant d'une femme non enceinte est testé, seule la ligne d'achèvement de test est observable visuellement ;
lorsqu'un échantillon provenant d'une femme présentant une grossesse normale est testé, la ligne d'achèvement de test, la ligne de détermination de grossesse extra-utérine et la ligne de détermination de grossesse normale sont observables visuellement avec des intensités égales ; et, lorsqu'un échantillon provenant d'une femme présentant une grossesse extra-utérine est testé, seule la ligne d'achèvement de test et la ligne de détermination de grossesse extra-utérine sont observables visuellement, ou bien la ligne d'achèvement de test, la ligne de détermination de grossesse extra-utérine et la ligne de détermination de grossesse normale sont observables visuellement, l'intensité de la ligne de détermination de grossesse normale étant inférieure à celle des lignes d'achèvement de test et de détermination de grossesse extra-utérine.

5. Dispositif de diagnostic de grossesse suivant l'une quelconque des revendications 1 à 4, dans lequel l'anticorps monoclonal anti-α-hCG et l'anticorps monoclonal anti-β-hCG ont été dissous dans un tampon d'immobilisation contenant 0,1 % à 2 % de saccharose et un tampon au phosphate 5-50 mM et ensuite immobilisés sur la membrane à raison de 0,1-5 μg/cm.

6. Dispositif de diagnostic de grossesse suivant l'une quelconque des revendications 1 à 5, dans lequel l'échantillon est choisi entre l'urine, le sang et la salive.

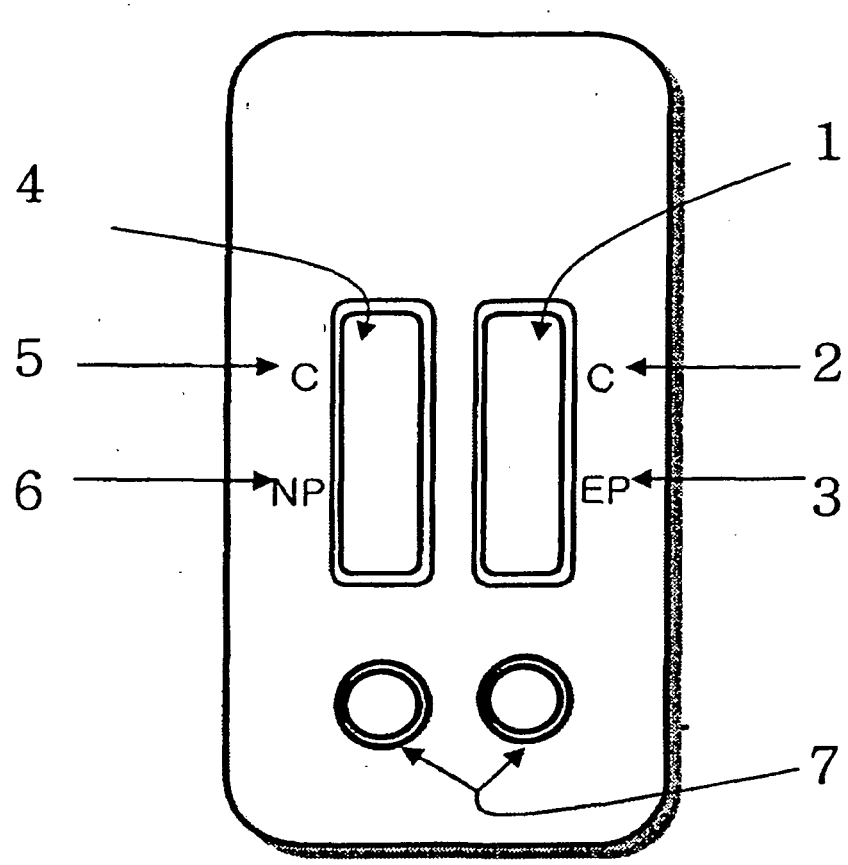

Fig. 1a

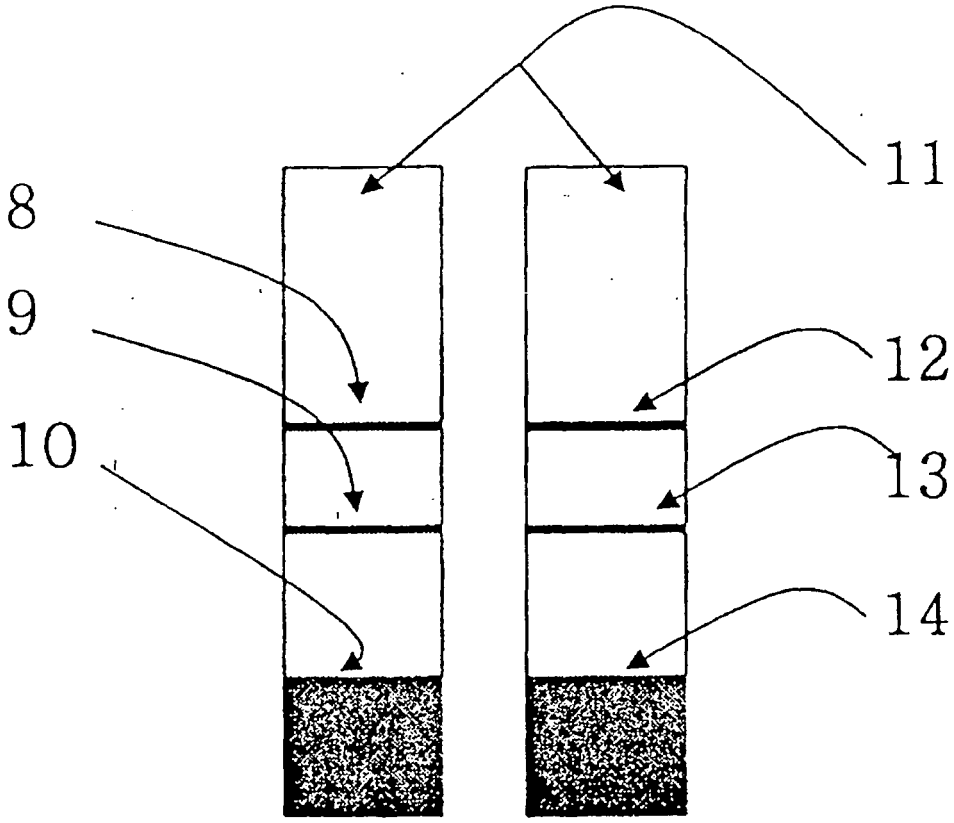

Fig. 1b

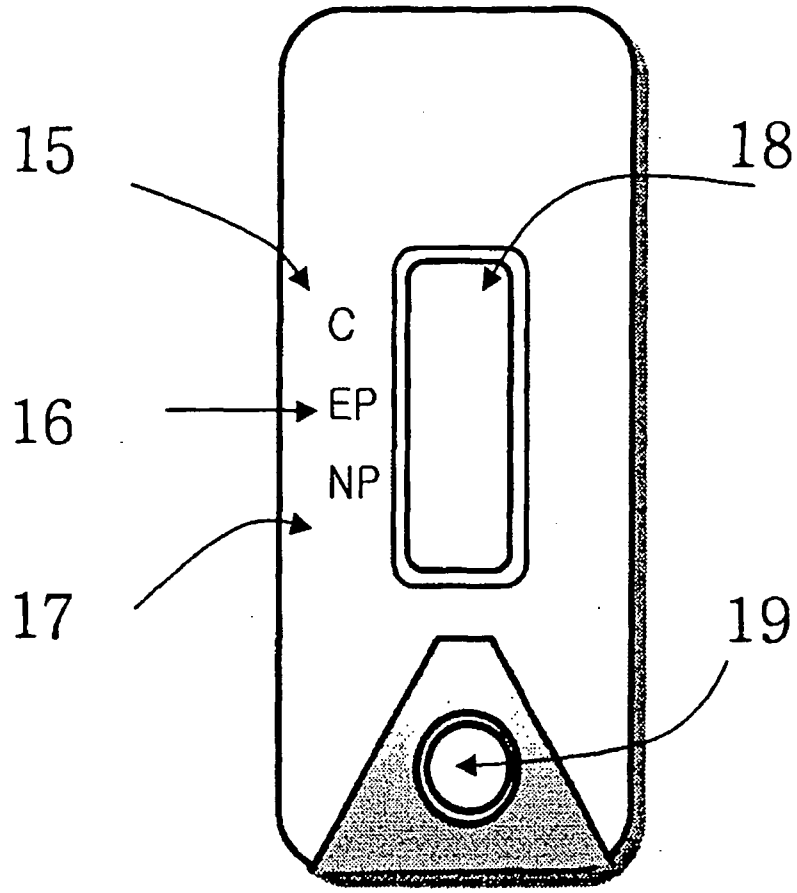

Fig. 2a

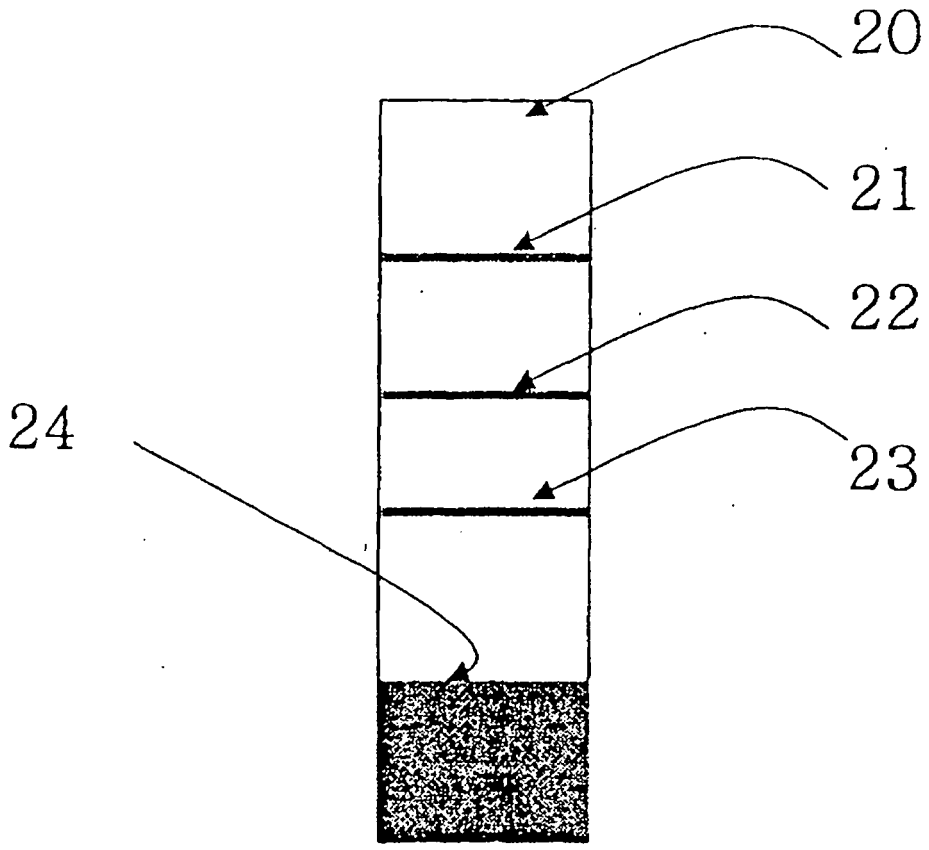

Fig. 2b

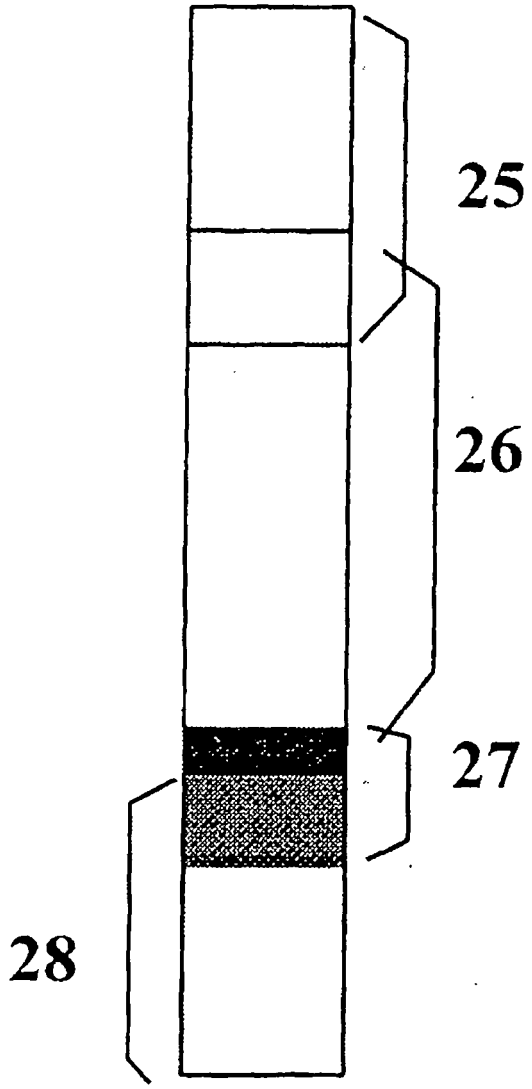

Fig. 3

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5786220 A **[0007] [0009]**

Non-patent literature cited in the description

- **LAPTHORN et al.** *Nature,* 1994, vol. 369, 455-461 **[0004]**
- **DENIL et al.** *Fertility & Sterility,* 1999, vol. 72, 1013-1017 **[0007]**
- **COLE et al.** *The Journal of Clinical Endocrinology and Metabilism,* 1994, vol. 78 (2), 497-499 **[0010]**
- **GALFRE, G. et al.** *Methods Enzymol.,* 1981, vol. 73, 3-46 **[0028]**

专利名称(译)	用于区分正常和异位妊娠的诊断装置		
公开(公告)号	EP1307134B1	公开(公告)日	2013-04-10
申请号	EP2001957025	申请日	2001-08-10
[标]发明人	CHANG JIN DONG CHA JUNG HAK NAM JUNG HYUN		
发明人	CHANG, JIN-DONG CHA, JUNG-HAK NAM, JUNG-HYUN		
IPC分类号	G01N33/558 G01N33/68 G01N33/76 G01N33/53 A61B5/00 A61B8/08 A61B10/00 G01N33/50 G01N33/531 G01N33/543 G01N33/577		
CPC分类号	G01N33/76 A61B8/0866 A61B10/0012 G01N33/558 G01N33/689 G01N2800/368 Y10S436/818		
优先权	1020000046755 2000-08-12 KR		
其他公开文献	EP1307134A1 EP1307134A4		
外部链接	Espacenet		

摘要(译)

公开了一种用于同时检测和区分正常妊娠和异位妊娠的一步诊断装置以及制备该装置的方法。利用本发明的装置和原理，通过免疫学检测人绒毛膜促性腺激素（hCG）与其分泌到体液中的其修饰形式之间的形态差异，可以在早期快速准确地确定正常妊娠和异位妊娠。怀孕的女性。

Table 3. Determination of clinical accuracy of the simultaneous diagnosis kit

Specimen / Type		1	2	3	4	5	6	7	8	9	10
Urine (non-pregnant)	N	-	-	-	-	-	-	-	-	-	-
	P										
	EP	-	-	-	-	-	-	-	-	-	-
Urine (normal pregnancy)	N	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	P										
	EP	+++	+++	+++	+++	++	+++	+++	++	+++	+++
Urine (ectopic pregnancy)	N	+	-	+	+	-	-	+	-	-	+
	P										
	EP	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Notes: - (no reaction), + (very little reaction), +++ (strong reaction)