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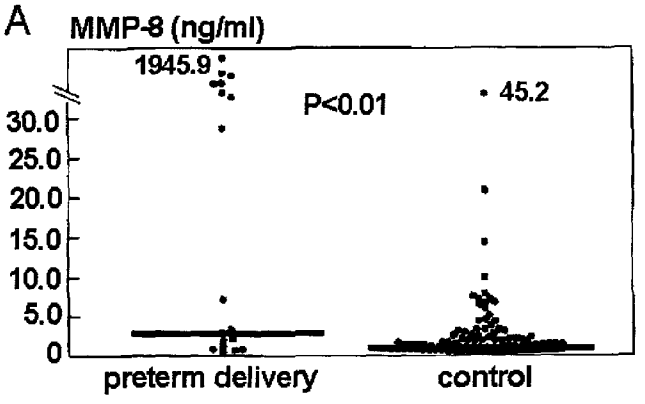

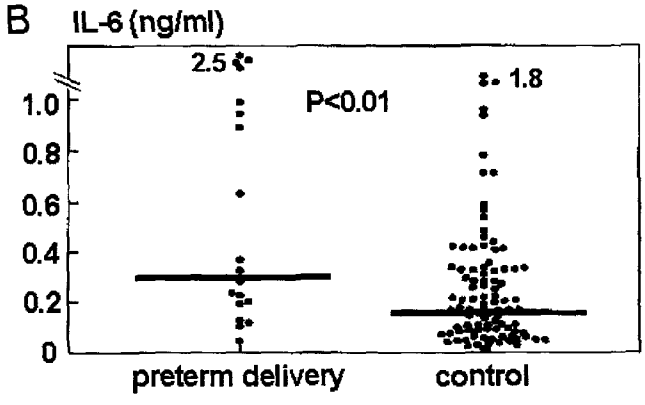

(57) **Abstract:** This invention is about a method for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage, and diagnostic reagent system and diagnostic kit for the diagnosis. The method, diagnostic reagent system, and kit are based on the finding that the level of MMP-8 in the amniotic fluid is significantly higher when the pregnant woman is at risk for preterm delivery, intrauterine infection, and fetal damage. The diagnostic reagent system and kit can be applied to the patients without, as well as with, clinical signs of preterm labor or premature rupture of fetal membranes. With the superiority in sensitivity and specificity as well as its less invasiveness compared to the conventional method of measuring fetal blood cytokine levels, this diagnostic reagent system and kit is very useful in the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage.

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DIAGNOSTIC AGENTS FOR THE PRENATAL DIAGNOSIS OF PRETERM DELIVERY, FETAL INFECTION, AND FETAL DAMAGE, AND DIAGNOSTIC KIT CONTAINING THE SAME

FIELD OF THE INVENTION

The present invention relates to a diagnostic reagent system for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage, and a diagnostic kit using the same reagents and, more particularly, to the use of an amniotic fluid matrix metalloproteinase-8 (MMP-8) concentration as a prenatal diagnostic marker for preterm delivery, fetal infection, and fetal damage.

BACKGROUND OF THE INVENTION

It has long been recognized in the medical world that the prevention of preterm delivery or premature rupture of fetal membranes is preferred to the post-treatment thereof. However, a great variety of factors are now known to cause preterm delivery or premature rupture of fetal membranes, making it difficult to prevent the undesirable events. Traditional approach to the prevention of preterm delivery was to identify high-risk group of women to which special attention should be paid based on the knowledge of

obstetrics and gynecology, demography, and various syndromes (Main et al., Am. J. Obstet. Gynecol., 151:892-898, 1985). However, this approach has the problem of being neither sensitive nor specific. To circumvent this problem, extensive research has been directed to find biochemical markers for the prediction of impending preterm delivery and premature rupture of fetal membranes, leading to the nomination of plasma estradiol-17 beta, progesterone, C-reactive protein as promising candidates. However, these candidates were found to be of poor accuracy.

Besides the identification of such biochemical predictable markers, significant attention has been paid to the biochemical role of collagen, based on the fact that the chorionic membrane is composed of fibrous connective tissue and the tensile strength of fibrous connective tissue is determined by its collagen content, as revealed through studies on the premature rupture of fetal membranes. On the basis of their finding that prematurely ruptured fetal membranes has low collagen content compared to normal membranes, some scientists concluded that the premature rupture of fetal membranes is attributable to the lower tensile strength than that of normal fetal membranes (Obstet. Gynecol., 57:487-89, 1981). According to another study, it was reported that the serum activity of collagenase was high in prematurely ruptured fetal membranes and preterm labor

(Obstet. Gynecol., 75:84-88, 1990). However, the precise mechanism of such biochemical changes has not yet been elucidated (FEBBS Letters, 244(2):315-318, 1989).

Statistically, the frequency of preterm delivery before 37 weeks of gestation is estimated to be about 8 to 10%. In Korea, about 50,000 neonates are prematurely delivered every year. Preterm delivery often causes serious neonatal complications including sepsis, respiratory distress syndrome, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis and cerebral palsy. The earlier the preterm delivery is, the greater is the frequency and severity of such sequelae. Therefore, if preterm delivery is prevented, it will be possible to reduce remarkably the occurrence of premature neonates disabled by such diseases.

Recent reports disclose that at least 30 to 40% of preterm deliveries are associated with intrauterine infection (Butler NR., Bonham DG., Prenatal mortality. The first report of the British perinatal mortality survey, Edinburgh, Churchill Livingstone, 115-145, 1963; Romero R., Avila C., Sepulveda W., Preterm birth. Cause, prevention, and management., McGrow-Hill Company, 97-136, 1993; Romero R., Mazor M., Clin. Obstet. Gynecol., 31:553, 1990; Gibbs RS., Romero R., Hiller SL., et al., Am. J. Obstet. Gynecol., 166:1515, 1992).

Intrauterine infection may cause fetal damage by the following process. Intrauterine infection activates the maternal and fetal immune system to secrete inflammatory mediators, such as cytokines from lymphocytes and MMPs (matrix-metalloproteinases) from neutrophils. When the inflammatory mediators reach a certain level, prostaglandin, which promotes uterine contraction, is produced, causing active labor leading to preterm delivery. Additionally, increased levels of inflammatory mediators cause the fetus to be affected by fetal inflammatory response syndrome (FIRS). Inflammatory mediators cause sepsis or acute respiratory distress syndrome or damage organs as a result of autoimmune diseases in adults, likewise organs of the fetus can be systemically injured by inflammatory mediators, resulting in brain white matter lesions and bronchopulmonary dysplasia. Therefore, the prenatal diagnosis of intrauterine inflammation is essential for the prevention of preterm delivery and fetal damage.

Generally, the prenatal diagnostic methods of intrauterine fetal infection in current use are cordocentesis, by which fetal blood cytokine levels are measured, and histologic examination of the umbilical cord to identify funisitis. However, cordocentesis is limitedly used due to its invasiveness, and funisitis can be diagnosed only after delivery (Yoon BH., Romero R., Park JS., Kim CJ.,

Choi JH., Han TR., Am. J. Obstet. Gynecol., 182:675-81, 2000; Yoon BH., Romero R., Kim KS., Park JS., Ki SH., Kim BI., Jun JK., Am. J. Obstet. Gynecol., 181:773-9, 1999; Romero R., Gomez R., Ghezzi F., Yoon BH., Mazor M., Edwin SS., Berry SM., Am. J. Obstet. Gynecol., 179:186-93, 1998).

The white blood cell count in the amniotic fluid is increased in cases of infection or inflammation of the amniotic cavity. Neutrophils in the amniotic fluid are considered to be of fetal origin (Knauper V., Kramer S., Reinke H., Tschesche H., Eur. J. Biochem., 189:295-300, 1990; Blaser J., Triebel S., Massjosthusmann U., Romisch J., Krahl-Mateblowski U., Freudenberg W., Fricke R., Tschesche H., Clinic. Chim. Acta., 244:17-33, 1996; Segura-Valdez L., Pardo A., Gaxiola M., Uhal BD., Becerril C., Selman M., Chest.., 117:684-94, 2000; Romanelli R., Mancini S., Laschinger C., Overall CM., Sodex J., MaCulloch CA., Infect. Immun., 67:2319-26, 1999; Maymon E., Romero R., Pacora P., Gomez R., Athayde N., Edwin S., Yoon BH., Am. J. Obstet. Gynecol., 183:94-9, 2000). Therefore, it is postulated that secretory products of neutrophils in amniotic fluid might reflect a fetal inflammatory response. In the present invention, the level of MMP-8 in the amniotic fluid is focused on. The determination of MMP-8 in amniotic fluid may be a marker of the fetal inflammatory response syndrome which can be diagnosed currently by histologic examination

of the umbilical cord after delivery or by cordocentesis with the determination of fetal blood cytokines.

MMP (matrix metalloproteinase) series, also collectively known as matrixins, are zinc-dependent endopeptidases that function to degrade extracellular matrix proteins. These proteases constitute a large and growing family of proteins which share similar structures and enzymatic properties. MMPs are broadly classified into five groups. Along with MMP-1 and MMP-13, MMP-8 belongs to an interstitial collagenase group. MMP-8 is similar in size to other interstitial collagenases, but is glycosylated to a far greater extent. When fully glycosylated, a proenzyme form of MMP-8 has a molecular weight of 85 kDa. The proenzyme is converted into an active form of 60-70 kDa with the loss of 15-25 kDa segment. ProMMP-8 is activated *in vitro* by various proteinases, including trypsin, chymotrypsin and cathepsin G. Organomercurial compounds were also found to activate proMMP-8. The activation mechanism of MMP-8 in vivo has not yet been fully clarified.

The prior technique concerning MMP-8 is found in U. S. Pat. No. 5,736,341 which discloses methods and test kits capable of sensitive and specific diagnosis of periodontal diseases, based on monoclonal antibodies against MMP-8. Described in the patent is that, since MMP-8 is directly associated with the destruction of periodontal connective

tissues during progression of periodontitis and diffused into oral cavity through the gingival pocket containing gingival crevicular fluid, measurement of the MMP-8 level in the oral cavity enables the site-specific diagnosis of periodontitis. For the specific and sensitive biochemical detection of periodontal diseases in progression, these methods measure the conversion of a proform of MMP-8 into an active form, because the conversion takes place during the process of periodontal infection. Nowhere is mentioned the use of MMP-8 in connection with preterm delivery and fetal infection and damage.

U. S. Pat. No. 5,641,636 refers to a method of predicting the onset of fetal membrane rupture based on the activity of another matrix collagenase, MMP-9, which belongs to a different group of enzymes from that of MMP-8. MMP-9 is a 92-kDa type IV collagenase/gelatinase or gelatinase B with the largest molecular weight among MMPs. For activation, the proenzyme form of MMP-9, i.e. proMMP-9, is initially cleaved into an intermediate active form of about 83 kDa with concurrent production of a 9 kDa inactive cleavage fragment. The intermediate active form is further processed proteolytically into an active form of MMP-9 with 67 kDa (J. Biol. Chem., 267 (30):21712-21719, 1992). The activation of MMP-9 means the conversion into the 83 kDa intermediate active form or the 67 kDa fully active form with gelatin

degradation activity. This patent measures the hydrolytic activity of MMP-9 to degrade denatured collagens, e.g. gelatins, to diagnose the premature rupture of fetal membranes. However, because MMP-9 is already present in the amniotic fluid before the onset of parturition, this method has limited value in predicting the premature rupture of fetal membranes.

Approximately 30 to 40% of patients with preterm labor or premature rupture of fetal membranes undergo preterm delivery. In this condition, various substances, including interleukin-6, interleukin-8, TNF-α, interleukin-1β, GROα, RANTES, white blood cells, MIP-1α, MCP-1, glucose, PGE_2, and angiogenin, are known to be present at increased levels in the amniotic fluid. However, these substances are of poor utility for the prediction of preterm delivery because their levels remain unchanged or are not detected in the amniotic fluid of the pregnant women without clinical signs of preterm labor and are increased only after the onset of preterm labor or premature fetal membrane rupture.

SUMMARY OF THE INVENTION

The intensive and thorough research on the prediction of preterm delivery, fetal infection, and fetal damage, conducted by the present inventors to overcome the problem

of invasiveness of cordocentesis with the determination of fetal blood cytokines and the problem of post-delivery identification of funisitis through histologic examination of the umbilical cord, resulted in the finding that the activity of MMP-8 is detected in the amniotic fluid of women without clinical signs of preterm labor or preterm premature rupture of fetal membranes as well as those with such signs, which allowed the development of a method and kit capable of diagnosing preterm delivery, fetal infection, and fetal damage.

Therefore, it is the objective of the present invention to provide a method and kit for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage in pregnant women with or without clinical signs of preterm labor or premature rupture of fetal membranes, whereby neonatal morbidity and serious complications or sequelae, such as cerebral palsy, can be prevented.

As one element of the present invention, there is provided a method for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage, by measuring the level of MMP-8 in the amniotic fluid in pregnant women with or without clinical signs of preterm labor or premature rupture of fetal membranes.

As another element of the present invention, there is provided a diagnostic reagent system and kit for the

prenatal diagnosis of preterm delivery, fetal infection, and fetal damage.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows distributions of amniotic fluid MMP-8 and IL-6 concentrations in patients with a spontaneous preterm delivery and with a spontaneous full-term delivery.

Fig. 2 shows receiver operating characteristic curves in which sensitivity is plotted versus specificity to select a cutoff value for amniotic fluid MMP-8 in the identification of spontaneous preterm delivery.

Fig. 3 shows distributions of amniotic fluid MMP-8 concentrations in patients with and without funisitis.

Fig. 4 shows a receiver operating characteristic curve in which sensitivity is plotted versus specificity to select a cutoff value for MMP-8 in the identification of funisitis.

Fig. 5 shows distributions of amniotic fluid MMP-8 concentrations in patients with and without prenatal development of cerebral palsy.

Fig. 6 shows a receiver operating characteristic curve in which sensitivity is plotted versus specificity to select a cutoff value for MMP-8 in the identification of prenatal development of cerebral palsy.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, the present invention pertains to determination of the activity of MMP-8 in the amniotic fluid, thereby prenatally diagnosing preterm delivery, fetal infection, and fetal damage.

With activity to degrade extracellular matrix proteins, MMP-8 is a zinc-dependent endopeptidase. Belonging to an interstitial collagenase group, MMP-8 can be purified from neutrophilic leukocytes in proenzyme form (proMMP-8). ProMMP-8 is highly glycosylated with a molecular weight of approximately 85 kDa. Activation of the proenzyme is achieved by cleavage of the proenzyme molecule, which creates the active collagenase whose molecular weight varies from 60 to 70 kDa, depending on the mode of activation. The mechanism of activation in vivo likely involves reactive oxygen species and oxidants such as hydroxyl radicals. When the N-terminal segment of the procollagenase are removed, the active site of the enzyme is generated and exposed.

MMP-8 is produced as a proenzyme form (proMMP-8) by neutrophilic leukocytes. ProMMP-8 exists in granules for storage and is secreted in response to stimuli. Secreted proMMP-8 is activated at extracellular interstitium and degrades type I, II and III collagens with high specificity for type I collagen. This enzyme, known as an important

mediator of inflammation-related tissue injury, is involved in the tissue injury caused by inflammatory diseases such as periodontitis, chronic obstructive pulmonary disease, rheumatoid arthritis, etc. Also, MMP-8 is known to be implicated in the progression of labor by causing the effacement and dilatation of the uterine cervix.

Based on the finding that an elevated mid-trimester amniotic fluid concentration of MMP-8 in women both with and without clinical signs of preterm labor or premature rupture of fetal membranes, is highly indicative of preterm delivery, the present inventors developed a prenatal diagnostic method of preterm delivery, fetal infection, and fetal damage.

In accordance with an embodiment of the present invention, there is provided a method for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage, comprising the steps of:

1) sampling an amniotic fluid from a pregnant woman; and

2) quantitatively measuring MMP-8 in the amniotic fluid sample.

Not only women with clinical signs of preterm labor or premature rupture of fetal membranes, but also women without such clinical signs can be diagnosed for preterm delivery.

The sampling of an amniotic fluid can be achieved by transabdominal amniocentesis under ultrasonographic guidance

or other sampling processes. For quantitative determination of an MMP-8 level in the amniotic fluid, any analytic methods, if based on antigen-antibody coupling, can be used, and ELISA (enzyme-linked immunosorbent assay) is preferred.

To determine if MMP-8 serves as a powerful clinical indicator for the prediction of preterm delivery in a mid-pregnancy stage, amniotic fluid concentrations of MMP-8 were compared with those of IL-6, another excellent indicator of inflammation, in patients who had a spontaneous preterm delivery before 32 weeks of gestation or a term delivery after mid-trimester amniocentesis without any clinical signs of impending preterm delivery. The amniotic fluid concentrations of MMP-8 and IL-6 were significantly higher in those with preterm delivery. Patient with an amniotic fluid MMP-8 concentration higher than 23 ng/ml had a spontaneous delivery in 89% of cases, as seen in Fig. 1. In addition, statistical comparison demonstrated the superiority of MMP-8 to IL-6 in sensitivity and specificity for the prediction of preterm delivery. Considering sensitivity, specificity and odds ratio as a whole, an amniotic fluid MMP-8 level of 23 ng/ml was selected as a cutoff suitable for the prediction of preterm delivery.

An elevated mid-trimester amniotic fluid MMP-8 concentration higher than the cutoff means high risk of

preterm delivery before 32 weeks of gestation. Therefore, when genetic amniocentesis is conducted in a mid-pregnancy stage, the quantification of amniotic fluid MMP-8 levels is helpful in the identification of patients who are likely to undergo a preterm delivery.

To prove the usefulness of MMP-8 as a clinical predictor for fetal infection and fetal damage, amniotic fluids taken from consecutive patients who delivered preterm singleton neonates (gestational age < 36 weeks) within 72 hours of amniocentesis were cultured for aerobic and anaerobic bacteria and for mycoplasmas and amniotic fluid MMP-8 concentrations were determined by ELISA. A histologic examination of placenta was made after delivery. MMP-8 concentrations were observed to be significantly higher in patients with a positive amniotic fluid culture than in patients with a negative amniotic fluid culture. Also, the presence of histologic chorioamnionitis in placenta entailed a significantly higher amniotic fluid MMP-8 level than does the absence thereof (see data of Tables 1 to 4).

From these results, it can be inferred that the infection of amniotic fluid with microorganisms leads to such a significant increase in amniotic fluid MMP-8 level that quantification thereof can be diagnostic of intrauterine infection. As for histologic chorioamnionitis, it also induces a significant increase of amniotic fluid

MMP-8 level, so that the determination of the amniotic fluid MMP-8 level can reflect the intrauterine infection. In consequence, MMP-8 can be used as an effective clinical predictor for the prenatal diagnosis of intrauterine infection and inflammation as well as preterm delivery. Especially, quantification of amniotic fluid MMP-8 levels can diagnose intrauterine inflammation with higher specificity and positive predictive value.

In order to diagnose fetal infection straightforwardly, amniotic fluid concentrations of MMP-8 were compared according to the presence or absence of funisitis.

The fetal inflammatory response syndrome (FIRS) is a multi-system disorder associated with impending preterm delivery and adverse neonatal outcome. Regarded as the histologic counterpart of FIRS, inflammation of the umbilical cord (funisitis) has been associated with an increased risk for the development of cerebral palsy. Neutrophils found in the amniotic cavity are of fetal origin. Based on these findings, it is hypothesized that secretory products from neutrophils might be an index of FIRS. To test this hypothesis, the relationship between amniotic fluid matrix metalloproteinase-8 (MMP-8) and funisitis was examined. The MMP-8 concentration was observed to be significantly higher in the presence of funisitis than in the absence of funisitis. The diagnostic indices of MMP-8

in the identification of funisitis were found to be high in sensitivity, specificity, and negative predictive value (see Tables 5 and 6, and Figs. 3 and 4).

From these results, it was found that there is a strong association between amniotic fluid MMP-8 concentrations and fetal inflammation (funisitis). Therefore, the present invention proposes that assessment of amniotic fluid MMP-8 concentrations may assist the diagnosis of the fetal infection without resorting to conventional invasive fetal blood sampling.

With the evidence of the correlation between the amniotic fluid MMP-8 concentration and fetal infection, the present inventors studied the relationship between amniotic fluid MMP-8 concentrations and neonatal morbidity, which includes neonatal sepsis, respiratory distress syndrome, pneumonia, bronchopulmonary dysplasia, necrotizing enterocolitis, and intraventricular hemorrhage. A significantly higher MMP-8 concentration was seen in the presence of neonatal morbidity than in the absence of neonatal morbidity (see Table 7). The diagnostic indices of MMP-8 in the identification of neonatal morbidity were excellent in specificity, and positive and negative predictive values (see Table 7 and 8). Additionally, the amniotic fluid MMP-8 concentration was significantly higher in patients who delivered neonates with neonatal sepsis than

in those who delivered neonates without neonatal sepsis ($P<0.05$) (see table 9).

The data, taken together, demonstrates that amniotic fluid MMP-8 concentration can be useful as a diagnostic marker in the prediction of neonatal sepsis and perinatal morbidity.

Intrauterine infection or inflammation has been implicated in the etiology of cerebral palsy. FIRS is believed to cause fetal brain damage in term and preterm birth. Neutrophils are the cells most frequently recruited into the amniotic fluid in cases of infection and they are considered to be of fetal origin. The amniotic fluid level of MMP-8, an enzyme secreted by activated neutrophils, was found to be significantly higher in cases of intrauterine infection and/or inflammation, as demonstrated in the following Examples 2 and 3. Based on this background, an examination was made to determine if increased concentrations of matrix metalloproteinase-8 (MMP-8) in amniotic fluid are associated with the development of cerebral palsy at the age of three years.

A significantly higher level of MMP-8 was observed in the amniotic fluid taken from the patients whose newborns developed cerebral palsy, compared to those whose newborns did not develop cerebral palsy (see Fig. 5), demonstrating that MMP-8 is very useful as a diagnostic marker for the

prediction of cerebral palsy (see Figs. 5 and 6, and Table 11).

We could see that the elevated amniotic fluid MMP-8 concentration is associated with a six-fold higher odds of developing cerebral palsy. That is, the amniotic fluid MMP-8 concentration can be used as a prenatal diagnostic marker for the prediction of cerebral palsy.

After extensive studies, a cutoff value of amniotic fluid MMP-8 in the identification of preterm delivery, fetal infection, and fetal damage was selected. In this regard, the pregnant woman was identified as being at risk for preterm delivery, neonatal morbidity, fetal infection (funisitis), and cerebral palsy when cutoff value of amniotic fluid MMP-8 is higher than 5 - 100 ng/ml and preferably higher than 10 - 50 ng/.

Based on the usefulness of the amniotic fluid MMP-8 concentration as a diagnostic marker in the identification of preterm delivery, fetal infection, and fetal damage, a reagent system can be developed for diagnosing preterm delivery, fetal infection, and fetal damage.

Therefore, in another embodiment of the present invention, there is provided a diagnostic reagent system for the identification of preterm delivery, fetal infection, and fetal damage, which is based on the quantification of amniotic fluid MMP-8 concentrations. In detail, the

diagnostic reagent system makes use of an analytic mechanism comprising the steps of:

1) adsorbing primary MMP-8 antibodies onto a matrix,

2) incubating the MMP-8 antibodies adsorbed onto the matrix in the presence of an amniotic fluid and washing the matrix to remove unbound antigens,

3) coupling a secondary chromogenic enzyme- or fluorescent-linked antibody to the MMP-8 bound to the primary antibodies adsorbed onto the matrix, and

4) developing a chromogenic reaction in the matrix by use of a coloring agent with quantitative analysis of the amount of antibody-bound MMP-8.

Examples of the matrix useful in the step 1 include a nitrocellulose membrane, a 96-well plate of polyvinyl resin, a 96-well plate of polystyrene resin, and a glass slide.

For quantitative analysis, the secondary antibody to be coupled to the MMP-8 bound to the primary antibody is linked to a chromogenic enzyme, such as peroxidase, alkaline phosphatase, and biotin, or a fluorescent agent such as FITC (Fluorescein Isothiocyanate) and TRITC.

The coloring agent can be selected from a group of agents consisting of 4CN (4-chloro-1-naphtol), DAB (diaminobenzidine), AEC (aminoethyl carbazol), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), OPD (o-phenylenediamine) and TMB (tetramethyl benzidine).

In principle, the diagnosis is based on the quantification of amniotic fluid MMP-8 concentrations, which takes advantage of the reaction of MMP-8 to its antibodies. In this connection, monoclonal or polyclonal anti-MMP-8 antibodies are immobilized to a solid matrix and reacted to MMP-8 in a sample, followed by washing the matrix to remove unbound antibodies and MMP-8. Then, a secondary monoclonal or polyclonal antibody which is linked with an enzyme or a fluorescent is bound to the immobilized MMP-8. A horseradish peroxidase polyclonal antibody, or a biotinylated rabbit polyclonal or monoclonal antibody is usually used as the secondary antibody. A chromogenic reaction for visualization is developed in the presence of a peroxide and a coloring agent. Addition of an acid halts the chromogenic reaction, followed by the measurement of absorbance at 450 nm.

Based on this diagnostic mechanism, a diagnostic kit can be configured, which uses the same diagnostic reagent system.

Therefore, in a further embodiment of the present invention, there is provided a diagnostic kit for the identification and prediction of preterm delivery, fetal infection, and fetal damage. With the aid of the diagnostic kit, preterm labor or premature rupture of fetal membranes can be assessed quantitatively or qualitatively with

convenience. For the assay of an MMP-8 antigen, a marker antibody is prepared by coupling gold or colloid particles to an anti-MMP-8 antibody. The marker antibody against MMP-8 is bound to MMP-8 to form an immune complex. This immune complex is again reacted with the MMP-8 antibody, and then washing fluid for excess marker antibody usually made of urea etc. is added. A positive result can be visualized by the eye when the MMP-8 concentration exceeds a certain level. Such a diagnostic kit may comprise anti-MMP-8 antibody, standard MMP-8, matrix, assay buffer, chromogenic enzyme- or fluorescent-labeled secondary antibody, and adhesive plate cover.

Alternatively, the diagnostic kit of the present invention may adopt an automated analytic method using a biological microchip. For instance, a diagnostic kit can be structured to perform immunoblotting using an anti-MMP-8 antibody-coated slide glass. This diagnostic kit may comprise a biological microchip onto the surface of which an anti-MMP-8 antibody is immobilized, appropriate buffer, standard MMP-8, and secondary antibody.

EXAMPLES

A better understanding of the present invention may be obtained in light of the following examples which are set

forth to illustrate, but are not to be construed to limit the present invention.

EXAMPLE 1: Selection of Diagnostic Cutoff Value for Prenatal Diagnosis of Preterm Delivery

Strong evidences implicate chronic intra-uterine infection in the etiology of preterm delivery. In this example, an examination was conducted to determine if amniotic fluid concentrations of MMP-8 and interleukin-6 (IL-6) can be used to identify patients at risk for spontaneous preterm delivery among pregnant women without clinical signs of preterm delivery.

For this end, a case-control study was designed with stored amniotic fluid obtained from women who had mid-trimester genetic amniocentesis. Amniotic fluid levels of MMP-8 and IL-6 were determined by ELISA in 19 patients with a spontaneous preterm delivery before 32 weeks of gestation and 95 control cases who delivered normal neonates at full-term. Cases with an abnormal fetal karyotype and major anomalies were excluded from this analysis.

The median amniotic fluid MMP-8 concentration with spontaneous preterm delivery was 3.1 [0.3-1954.9] ng/ml, while the level was 1.3 [<0.3-45.2] ng/ml in the control cases. The median amniotic fluid IL-6 concentration was 0.32

[0.04-2.52] ng/ml in patients with spontaneous preterm delivery while the level was 0.18 [0.01-1.81] ng/ml in the control cases. Both MMP-8 and IL-6 concentrations of amniotic fluid taken at mid-pregnancy stage were significantly higher in patients with spontaneous preterm delivery than in the control cases with full-term delivery ($p<0.01$), as shown in Fig. 1.

After the assessment of MMP-8 concentrations in patients and the control cases, when the amniotic fluid MMP-8 concentration at a mid-pregnancy stage was above 23 ng/ml, 89% of the cases investigated had a spontaneous preterm delivery.

Additionally, an amniotic fluid MMP-8 cutoff value of 23 ng/ml at a mid-pregnancy stage showed a sensitivity of 42% (8/19) and a specificity of 99% (94/95) in the identification of the patients with early preterm delivery after genetic amniocentesis, while an IL-6 cutoff value of 0.6 ng/ml showed a sensitivity of 42% (8/19) and a specificity of 92% (87/95), as shown in Fig. 2. Over IL-6, MMP-8 was therefore superior in sensitivity, specificity and odds ratio for the identification and prediction of preterm delivery. In consequence, an amniotic fluid MMP-8 concentration of 23 ng/ml was selected as a cutoff in the identification of prematurity.

EXAMPLE 2: Relationship between Amniotic Fluid Concentration of MMP-8 and Intrauterine Infection and Inflammation

Amniotic fluid concentrations of MMP-8 were examined in the presence or absence of intrauterine infection and inflammation to determine if the amniotic fluid MMP-8 concentration could be used as a diagnostic marker in the identification of intrauterine infection and fetal damage.

In this regard, 255 consecutive patients who delivered preterm singleton neonates (gestational age < 36 weeks) within 72 hours of amniocentesis at Seoul National University Hospital, Seoul, Korea, were examined. The amniotic fluids were measured for MMP-8 level as well as cultured for aerobic and anaerobic bacteria and for mycoplasmas. In addition, histologic examination of the placenta was performed. Amniotic fluid was retrieved by transabdominal amniocentesis under ultrasonographic guidance.

2-1: Amniotic Fluid Culture

Immediately after being retrieved by transabdominal amniocentesis, the amniotic fluid was put into sterile plastic vessels with caps, and stored therein until culturing in anaerobic or aerobic media. Useful in culturing aerobes or anaerobes were blood agar, McConkey's agar, Bactec 6A vial, thioglycollate broth, brucellar blood

agar, fresh meat extracts, and pleuropneumoni-like organism broth supplemented with horse serum penicillin polymixin B and amphotericin B. Mycotrim GU was used to culture mycoplasmas.

2-2: Measurement of Amniotic Fluid MMP-8 Concentration

Amniotic fluid samples were centrifuged at 700x g for 10 min. The supernatant was used to measure amniotic fluid MMP-8 concentration with ELISA (Amersham Pharmacia Biotech, UK) using two monoclonal antibodies which bind to non-overlapping epitopes.

2-3: Histologic Examination of Placenta

After the placenta was fully drawn out at delivery, tissues excised from the umbilical cord, the chorionic plate, and the placental membrane were fixed in 10% formalin and embedded in paraffin to prepare slides. Afterwards, the tissue segments were dyed with hematoxylin and eosin for visualization under a microscope. Acute intrauterine inflammation was defined as the presence of inflammatory changes on examination of any of the fetal membrane, chorionic membrane, decidua and the chorionic plate.

Of the 255 subjects, 45 cases were found to have positive amniotic fluid culture, which shows the intrauterine infection frequency to be 18%. On histologic

examination of the placenta 113 patients were found to have chorioamnionitis, which indicates the intrauterine inflammation frequency to be 44%. The median amniotic fluid MMP-8 concentrations were detected at a level of 191.4 ng/ml in patients with positive amniotic fluid culture of bacteria and at a level of 2.7 ng/ml in those with negative culture. Therefore, the median amniotic fluid MMP-8 concentration in patients with intrauterine infection were significantly higher than those without intrauterine infection ($p < 0.001$). The results are given in Table 1, below.

TABLE 1
Amniotic Fluid MMP-8 Concentration According to Amniotic Fluid Culture Result

Bacteria in Amniotic Fluid	Median Value(ng/mL)	Interval(ng/mL)
Positive	191.4	<0.3-4202.7
Negative	2.7	<0.3-3929.0

With a cutoff value of 23 ng/ml, the diagnostic indices of MMP-8 for the identification of positive amniotic fluid culture were excellent: sensitivity of 76% and negative predictive value of 93%. The results are given in Table 2, below.

TABLE 2
Diagnostic Indices of MMP-8 for the Identification of Positive Amniotic Fluid Culture

Sensitivity	76%
Specificity	70%
Positive Predictive Value	35%
Negative Predictive Value	93%

Histologic examination of the placenta revealed that the median concentration of amniotic fluid MMP-8 was 160.9 ng/ml in the presence of chorioamnionitis, but 1.0 ng/ml in the absence of chorioamnionitis. The amniotic fluid MMP-8 concentration was significantly higher in patients with chorioamnionitis than those without chorioamnionitis ($p<0.001$). The results are given in Table 3, below.

TABLE 3
MMP-8 Concentration According to Intrauterine Inflammation

Histologic Chorioamnionitis	Median Value(ng/mL)	Interval(ng/mL)
Present	160.9	<0.3-4202.7
Absent	1.0	<0.3-766.2

With a cutoff value of 23 ng/ml, the diagnostic indices of MMP-8 for the identification of intrauterine inflammation (chorioamnionitis) were excellent: sensitivity of 72%, specificity of 89%, positive predictive value of 84%, and negative predictive value of 80%. The results are given in Table 4, below.

TABLE 4

Diagnostic Indices for the Identification of Intrauterine Inflammation

Sensitivity	72%
Specificity	89%
Positive Predictive Value	84%
Negative Predictive Value	80%

EXAMPLE 3: Diagnosis of Funisitis By Use of Amniotic Fluid Concentration of MMP-8

Amniotic fluid MMP-8 concentrations were measured in the presence or in the absence of funisitis to determine if amniotic fluid MMP-8 concentrations could be utilized to directly diagnose fetal infection.

The relationship between the presence of funisitis and amniotic fluid concentrations of MMP-8 was examined in 255 consecutive patients who delivered preterm singleton neonates (gestational age < 36 weeks) within 72 hours of amniocentesis at the Seoul National University Hospital, Seoul, Korea. Funisitis was diagnosed by the presence of neutrophil infiltration into the umbilical vessel walls or Wharton jelly. Quantification of MMP-8 concentration was conducted in the same manner as in Example 2.

Funisitis was diagnosed in 59 cases (funisitis frequency 23%). The median amniotic fluid MMP-8

concentrations were 433.7 ng/ml in patients with funisitis and 1.9 ng/ml in those without funisitis. Therefore, the patients with funisitis had significantly higher median amniotic fluid MMP-8 concentrations compared to those without funisitis ($p < 0.001$). The results are given in Table 5, below and Fig. 3.

TABLE 5

MMP-8 Concentration According to Presence or Absence of Funisitis

Funisitis	Median Value(ng/mL)	Interval(ng/mL)
Present	433.7	1.5-3836.8
Absent	1.9	<0.3-4202.7

Receiver operating characteristic curve analysis was employed to select a cutoff value for amniotic fluid analytes in the diagnosis of funisitis. As a result, a cutoff of 23 ng/ml was selected for MMP-8 in consideration of both sensitivity and positive predictive value for the diagnosis of funisitis, as seen in Fig. 4. The diagnostic indices of MMP-8 (cutoff 23 ng/ml) in the identification of funisitis were excellent: sensitivity of 90%, specificity of 78%, and negative predictive value of 96%. The results are summarized in Table 6, below.

TABLE 6

Diagnostic Indices of MMP-8 for the Identification of Funisitis

Sensitivity	90% (53/59)
Specificity	78% (153/196)
Positive Predictive Value	55% (53/96)
Negative Predictive Value	96% (153/159)

EXAMPLE 4: Diagnosis of Neonatal Morbidity By Use of Amniotic Fluid MMP-8 Concentration

Based on the postulation that funisitis would be associated with an increased risk of neonatal infection-related complications such as sepsis, pneumonia, bronchopulmonary dysplasia, necrotizing enterocolitis, and intraventricular hemorrhage, amniotic fluid MMP-8 concentrations in cases with such neonatal morbidity were compared with those in normal cases.

The relationship between the presence of such neonatal morbidity and amniotic fluid concentration of MMP-8 was examined in 239 consecutive patients who delivered preterm singleton neonates (gestational age < 36 weeks) within 72 hours of amniocentesis at the Seoul National University Hospital, Seoul, Korea. Neonatal morbidity was defined by the development of neonatal complication, such as sepsis, pneumonia, bronchopulmonary dysplasia, necrotizing enterocolitis, and intraventricular hemorrhage. Congenital

neonatal sepsis was diagnosed in the presence of a positive blood culture within 72 hours of delivery. The diagnosis of neonatal respiratory distress syndrome required the presence of respiratory grunting and retracting, an increased oxygen requirement (FiO_2 > 0.4), and diagnostic radiographic and laboratory findings without the evidence of other causes of respiratory disease. Pneumonia was diagnosed in the presence of definite clinical and radiologic findings, with or without a positive culture from tracheal aspirate or chest tube specimen within 7 days of birth. Bronchopulmonary dysplasia was diagnosed by the criteria proposed by Bancalari et al.: (1) Intermittent positive-pressure ventilation was required during the first week of life and for a minimum of 3 days; (2) clinical signs of chronic respiratory disease developed, characterized by tachypnea, intercostal and subcostal retraction, and rales on auscultation, all persisting for longer than 28 days; (3) supplemental oxygen was required for more than 28 days to maintain a PaO_2 over 50 mmHg; (4) chest radiograph showed persistent strands of densities in both lungs, alternating with areas of normal or increased lucency. In some cases, an autopsy was needed for the diagnosis of bronchopulmonary dysplasia. Intraventricular hemorrhage was graded according to the system proposed by McMenamin et al. Necrotizing enterocolitis was diagnosed in the presence of abdominal

expansion and feeding intolerance for at least 24 hours with radiologic findings of air entrapment in enteric walls, intestinal rupture, and meconium obstructive syndrome, or by operational or autopsic findings of necrotic intestinal rupture. Quantification of amniotic fluid MMP-8 concentrations was conducted in the same manner as in Example 2.

107 neonates of the 239 subjects (frequency 45 %) were diagnosed to suffer neonatal morbidity. The median amniotic fluid MMP-8 concentration remained at a level of 2.35 ng/ml in the absence of major neonatal morbidity while a significantly higher MMP-8 concentration (160.9 ng/ml) was observed in the presence of major neonatal morbidity ($p<0.001$). Details are given in Table 7, below.

TABLE 7

MMP-8 Concentration According to the Presence or Absence of Neonatal Morbidity

Neonatal Morbidity	Median Value(ng/mL)	Interval(ng/mL)
Present	17.0	<0.3-4202.7
Absent	2.35	<0.3-1333.1

Receiver operating characteristic curve analysis was employed to select a cutoff value for amniotic fluid analytes in the diagnosis of neonatal morbidity. As a result, a cutoff of 23 ng/ml was selected for MMP-8

considering sensitivity and positive predictive value for the diagnosis of neonatal morbidity. The diagnostic indices of MMP-8 (cutoff 23 ng/ml) for the identification of neonatal morbidity were excellent: specificity of 77%, positive predictive value of 63% and negative predictive value of 65%. The results are summarized in Table 8, below.

TABLE 8

Diagnostic Indices of MMP-8 for the Identification of Neonatal Morbidity

Sensitivity	50%
Specificity	77%
Positive Predictive Value	63%
Negative Predictive Value	65%

Additionally, the median amniotic fluid MMP-8 concentration was significantly higher in the presence of sepsis (208.95 ng/ml), compared to the absence of sepsis (4.4 ng/ml) ($p<0.05$). Details are given in Table 9, below.

TABLE 9

MMP-8 Concentration According to the Presence or Absence of Neonatal Sepsis

Neonatal Sepsis	Median Value(ng/mL)	Interval(ng/mL)
Present	208.95	2.4-1568.6
Absent	4.4	<0.3-4202.7

The diagnostic indices of MMP-8 (cutoff 23 ng/ml) in

the identification of neonatal sepsis were excellent: sensitivity of 67%, specificity of 66%, and negative predictive value of 99%. The results are summarized in Table 10, below.

TABLE 10
Diagnostic Indices of MMP-8 for the Identification of Neonatal Sepsis

Sensitivity	67%
Specificity	66%
Positive Predictive Value	5%
Negative Predictive Value	99%

EXAMPLE 5: Diagnosis of Cerebral Palsy By Use of Amniotic Fluid MMP-8 Concentration

The relationship between amniotic fluid concentrations of MMP-8 and the development of cerebral palsy was examined in 116 preterm singleton newborns (gestational age at birth, < 35 weeks) born to mothers who underwent amniocentesis and were followed for at least 3 years. Cerebral palsy was diagnosed in the presence of a definite abnormality on the neurodevelopmental assessment (abnormality of developmental milestone, postural abnormality by the Vojta method, and reflex abnormality) and a persistent abnormality of muscle tone.

Median amniotic fluid concentration of MMP-8 was significantly higher in mothers whose newborns developed cerebral palsy than in mothers whose newborns did not develop cerebral palsy (median 153.9 [range <0.3-1535.9] ng /ml vs median 6.4 [range <0.3-3836.8] ng/ml; $p<0.01$). Neonates who developed cerebral palsy were delivered at earlier gestational age than those without cerebral palsy. After adjustment for the gestational age at birth and the results of amniotic fluid culture, elevated concentrations of amniotic fluid MMP-8 significantly increased the odds of development of cerebral palsy (odds ratio, 6.0; 95% confidence interval, 1.1-33.0; $p<0.05$).

Receiver operating characteristic curve analysis was employed to select a cutoff value for amniotic fluid analytes in the diagnosis of cerebral palsy. As a result, a cutoff of 23 ng/ml was selected for MMP-8 in consideration of both sensitivity and positive predictive value for the diagnosis of cerebral palsy, as shown in Fig. 6. The diagnostic indices of MMP-8 (cutoff 23 ng/ml) in the diagnosis of cerebral palsy were excellent: sensitivity of 85%, specificity of 69%, and negative predictive value of 97%. The results are summarized in Table 11, below.

TABLE 11

Diagnostic Indices of MMP-8 for Cerebral Palsy

Sensitivity	85%
Specificity	69%
Positive Predictive Value	26%
Negative Predictive Value	97%

INDUSTRIAL APPLICABILITY

As described hereinbefore, a diagnostic reagent system and a diagnostic kit are provided for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage, based on the quantification of amniotic fluid MMP-8 concentration. Taking advantage of an antigen-antibody reaction coupled with chromogenesis, the diagnostic reagent system and diagnostic kit of the present invention is characterized by comprising one or more of anti-MMP-8 antibody. The diagnostic reagent system and kit of the present invention can be applied to the patients without, as well as with, clinical signs of preterm delivery or premature rupture of fetal membranes. With the superiority in sensitivity and specificity to conventional method of measuring fetal blood cytokine levels, the present invention is very useful in the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage.

What is claimed is:

1. A method for the prenatal diagnosis of preterm delivery, fetal infection, and fetal damage, comprising the steps of:

1) sampling an amniotic fluid from a pregnant woman; and

2) measuring matrix metalloproteinase-8 level in the amniotic fluid sample.

2. The method as set forth in claim 1, wherein the pregnant woman is one with or without clinical signs of preterm labor or premature rupture of fetal membranes.

3. The method as set forth in claim 1, wherein the risk of preterm delivery, fetal infection, and fetal damage is prenatally diagnosed, including perinatal morbidity comprising neonatal sepsis, respiratory distress syndrome, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis, and cerebral palsy.

4. The method as set forth in claim 1, wherein the pregnant woman is identified as being at risk for preterm delivery, fetal infection, and fetal damage when cutoff

value of amniotic fluid matrix metalloproteinase-8 is higher than 5 - 100 ng/ml.

5. The method as set forth in claim 1, wherein the pregnant woman is identified as being at risk for preterm delivery when cutoff value of amniotic fluid matrix metalloproteinase-8 is higher than 10 - 50 ng/ml.

6. The method as set forth in claim 1, wherein the pregnant woman is identified as being at risk for fetal infection when cutoff value of amniotic fluid matrix metalloproteinase-8 is higher than 10 - 50 ng/ml.

7. The method as set forth in claim 1, wherein the pregnant woman is identified as being at risk for the development of cerebral palsy when cutoff value of amniotic fluid matrix metalloproteinase-8 is higher than 10 - 50 ng/ml.

8. The method as set forth in claim 1, wherein the pregnant woman is identified as being at risk for perinatal morbidity when cutoff value of amniotic fluid matrix metalloproteinase-8 is higher than 10 - 50 ng/ml.

9. A diagnostic reagent system utilizing the method of

claim 1, comprising one or more anti- matrix metalloproteinase-8 antibody.

10. The diagnostic reagent system as set forth in claim 9, wherein the diagnostic reagent system is based on the analytic mechanism comprising the steps of:

1) adsorbing primary anti-matrix metalloproteinase-8 antibodies onto a matrix,

2) incubating the anti-matrix metalloproteinase-8 antibodies adsorbed onto the matrix in the presence of an amniotic fluid and washing the matrix to remove unbound antigens,

3) coupling a secondary chromogenic enzyme- or fluorescent-linked antibody to the matrix metalloproteinase-8 bound to the primary antibodies adsorbed onto the matrix, and

4) developing a chromogenic reaction in the matrix by adding coloring agent with quantitative analysis of the specific antigen-antibody reaction.

11. The diagnostic reagent system as set forth in claim 10, wherein the anti-matrix metalloproteinase-8 antibodies are monoclonal or polyclonal.

12. The diagnostic reagent system as set forth in

claim 10, wherein the matrix is selected from a group of material consisting of a nitrocellulose membrane, a 96-well plate of polyvinyl resin, a 96-well plate of polystyrene resin, and a glass slide.

13. The diagnostic reagent system as set forth in claim 10, wherein the chromogenic enzyme is selected from a group of agents consisting of peroxidase, alkaline phosphatase and biotin.

14. The diagnostic reagent system as set forth in claim 10, wherein the fluorescent agent is selected from a group of agents consisting of FITC and TRITC.

15. The diagnostic reagent system as set forth in claim 10, wherein the coloring agent is selected from a group of agents consisting of 4-chloro-1-naphtol, diaminobenzidine, aminoethyl carbazole, 2,2'-azion-bis(3-ethylbenzothiazoline-6-sulfonic acid), o-phenylenediamine, and tetramethyl benzidine.

16. A diagnostic kit, comprising the diagnostic reagent system of claim 9.

17. The diagnostic kit as set forth in claim 16,

wherein the kit comprises anti-matrix metalloproteinase-8 antibody, standard matrix metalloproteinase-8, matrix, assay buffer, chromogenic enzyme- or fluorescent compound-linked secondary antibody, and adhesive plate cover.

FIGURES

FIG. 1

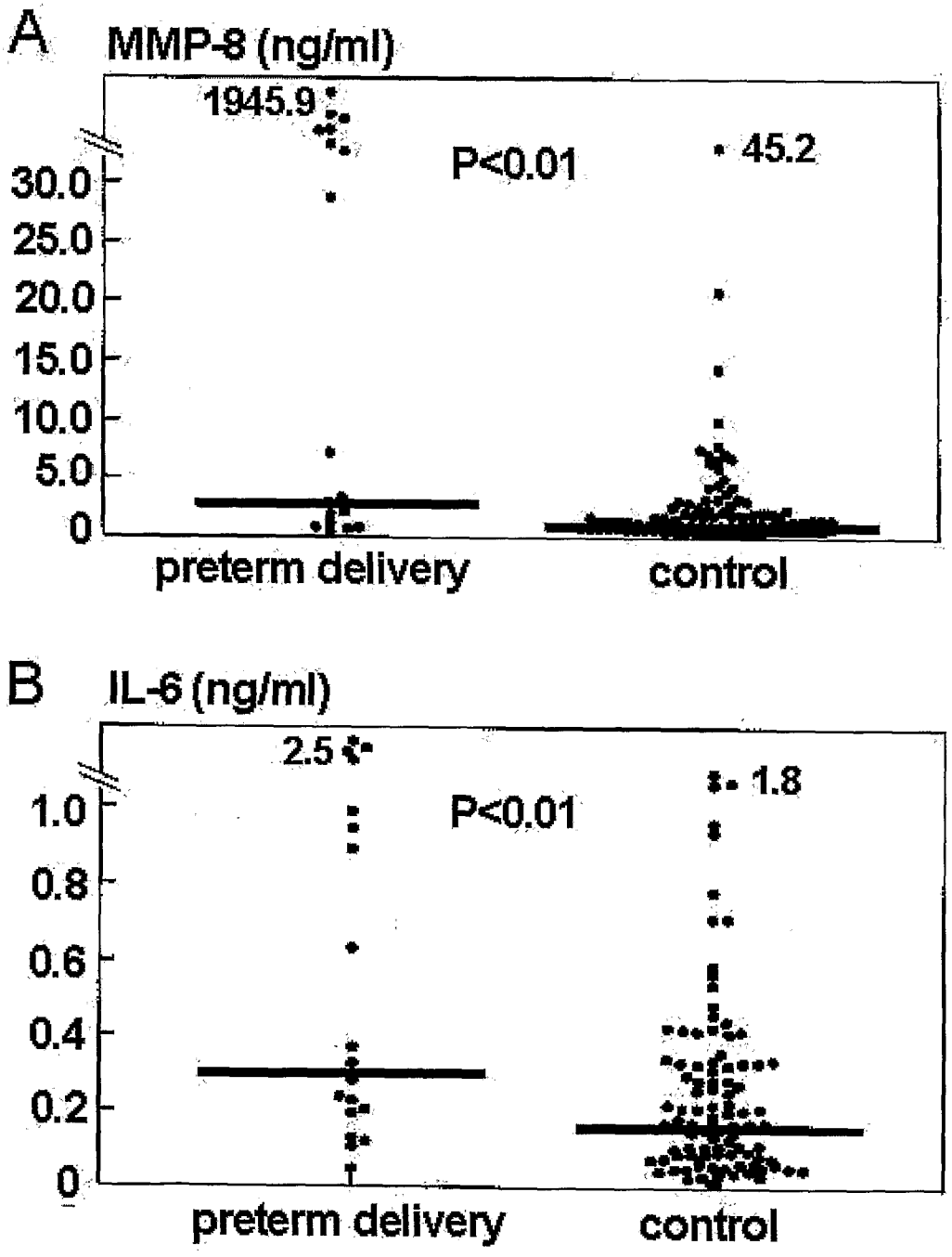


FIG. 2

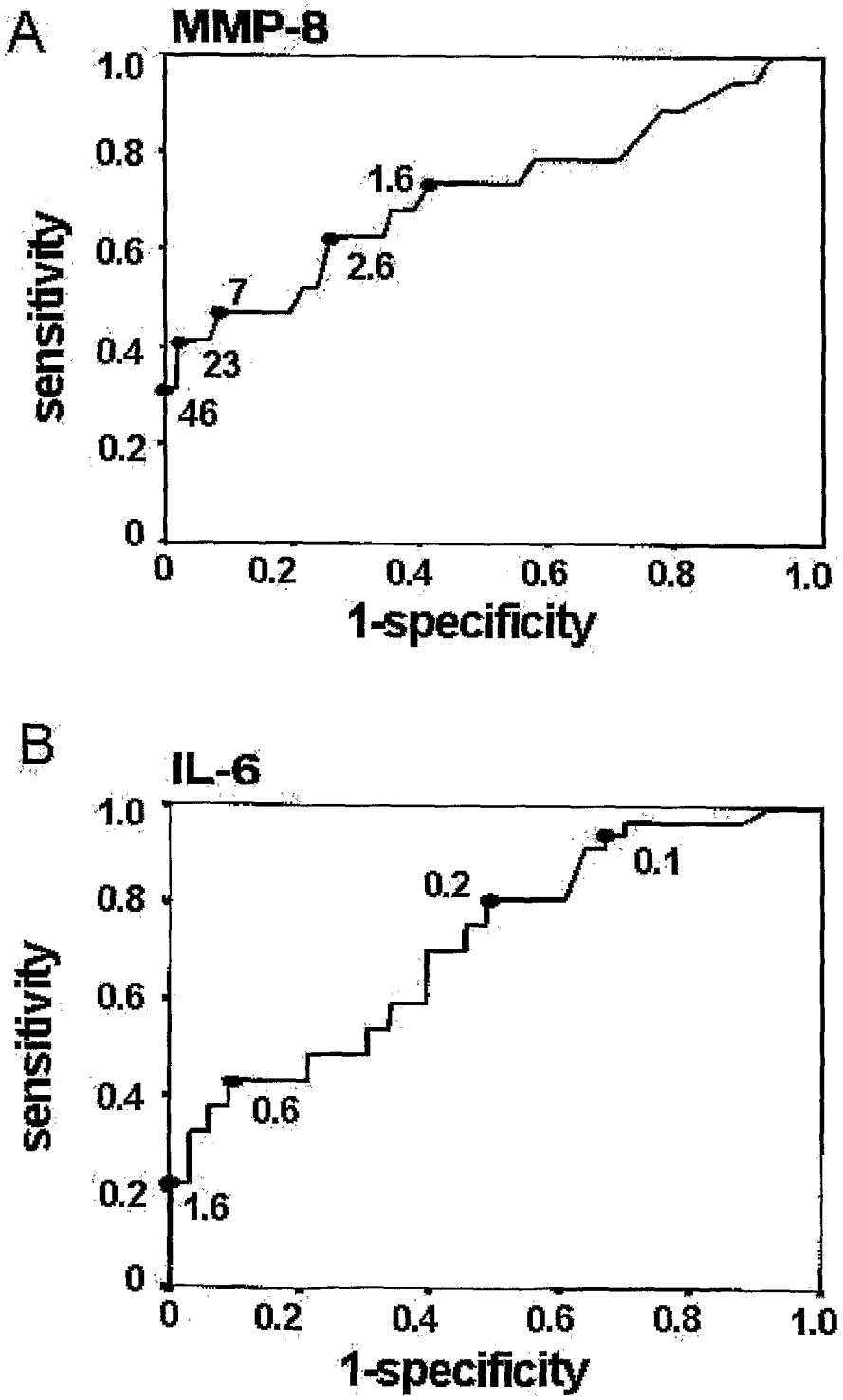
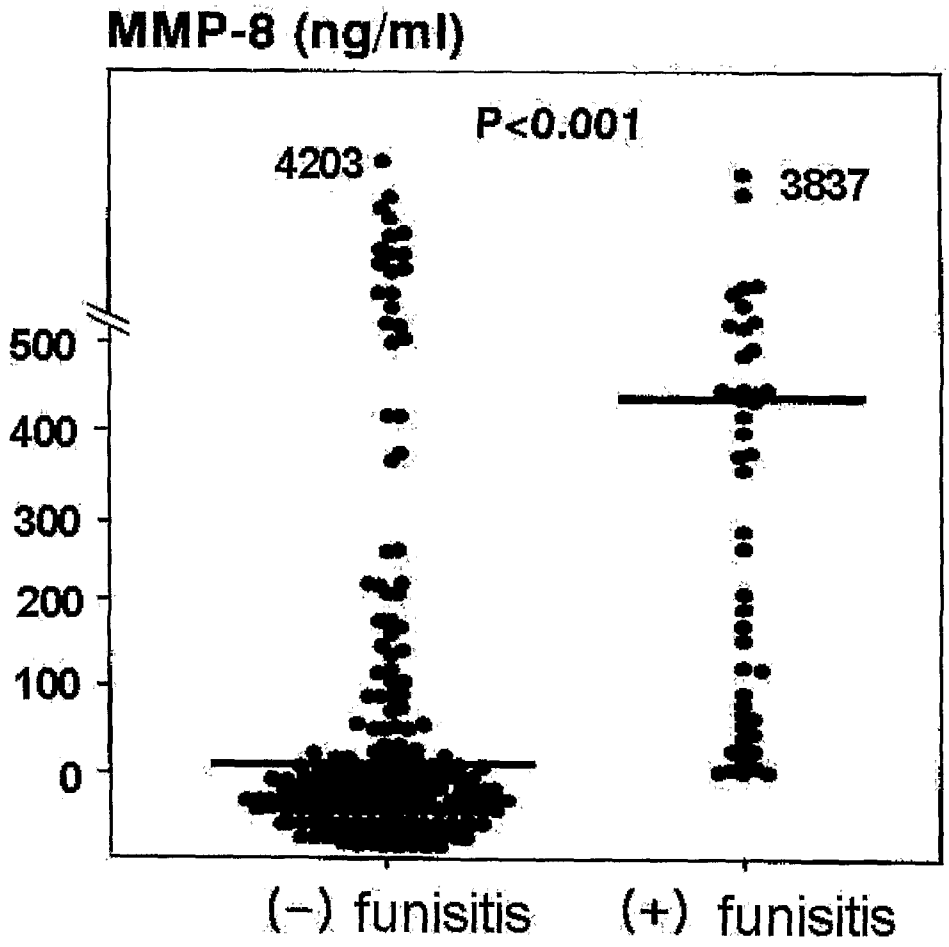

A
MMP-8
sensitivity
1-specificity
1.6
2.6
7
23
46
B
IL-6
sensitivity
1-specificity
0.1
0.2
0.6
1.6

FIG. 3


MMP-8 (ng/ml)
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3837
500
400
300
200
100
0
(-) funisitis
(+) funisitis

FIG. 4

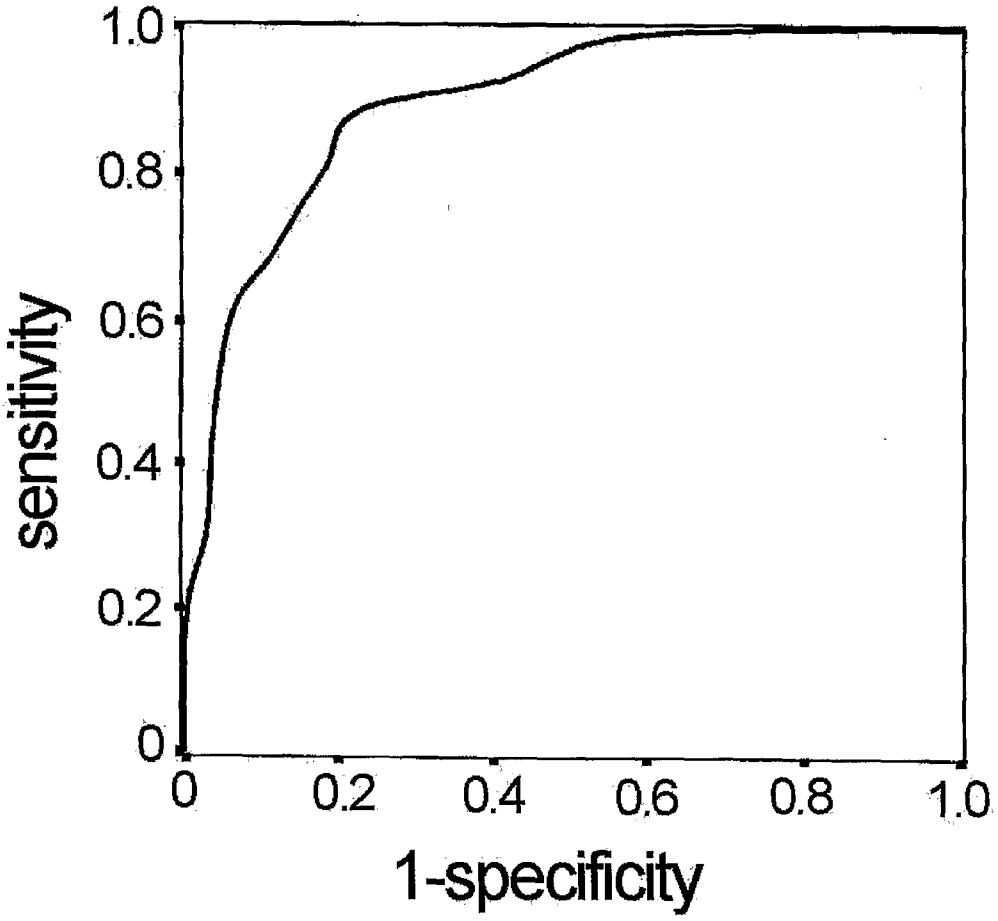


FIG. 5

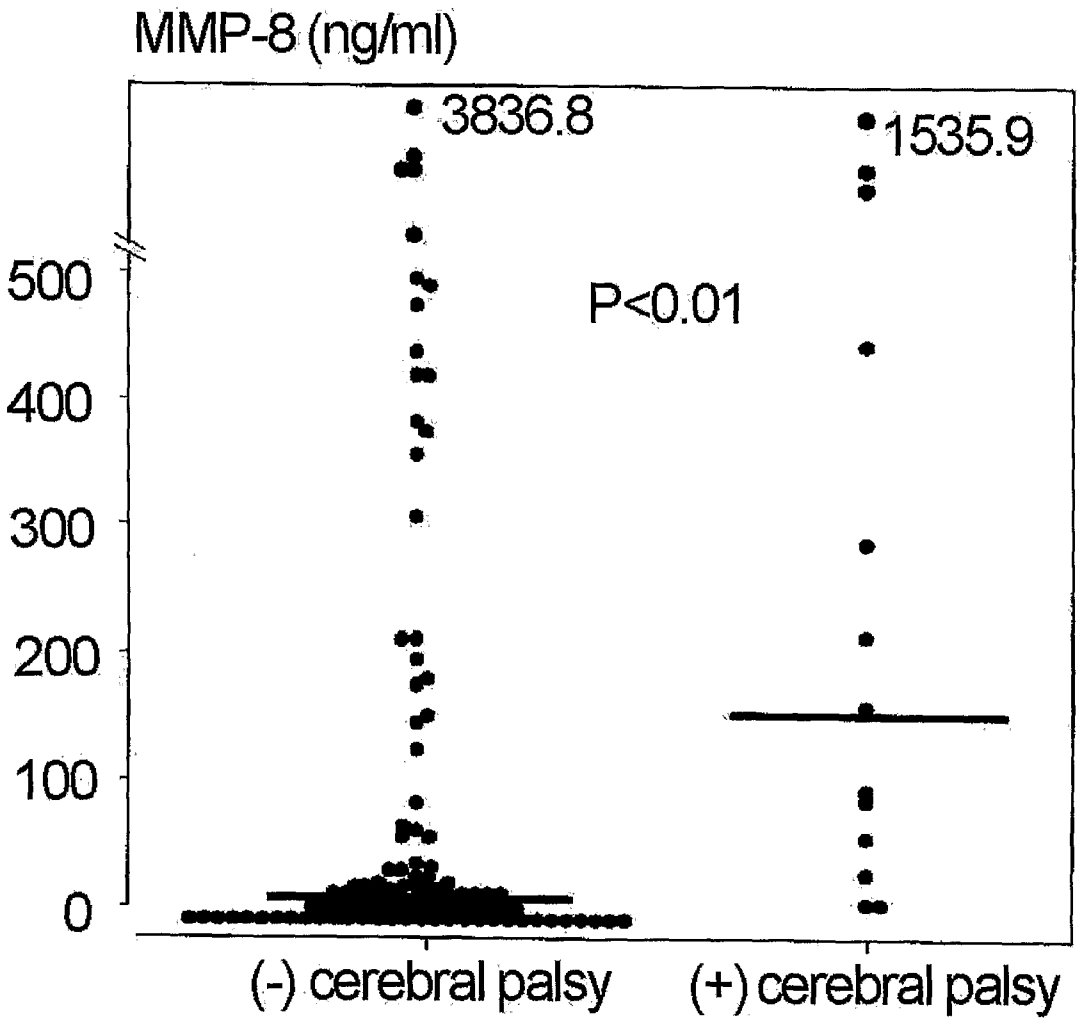
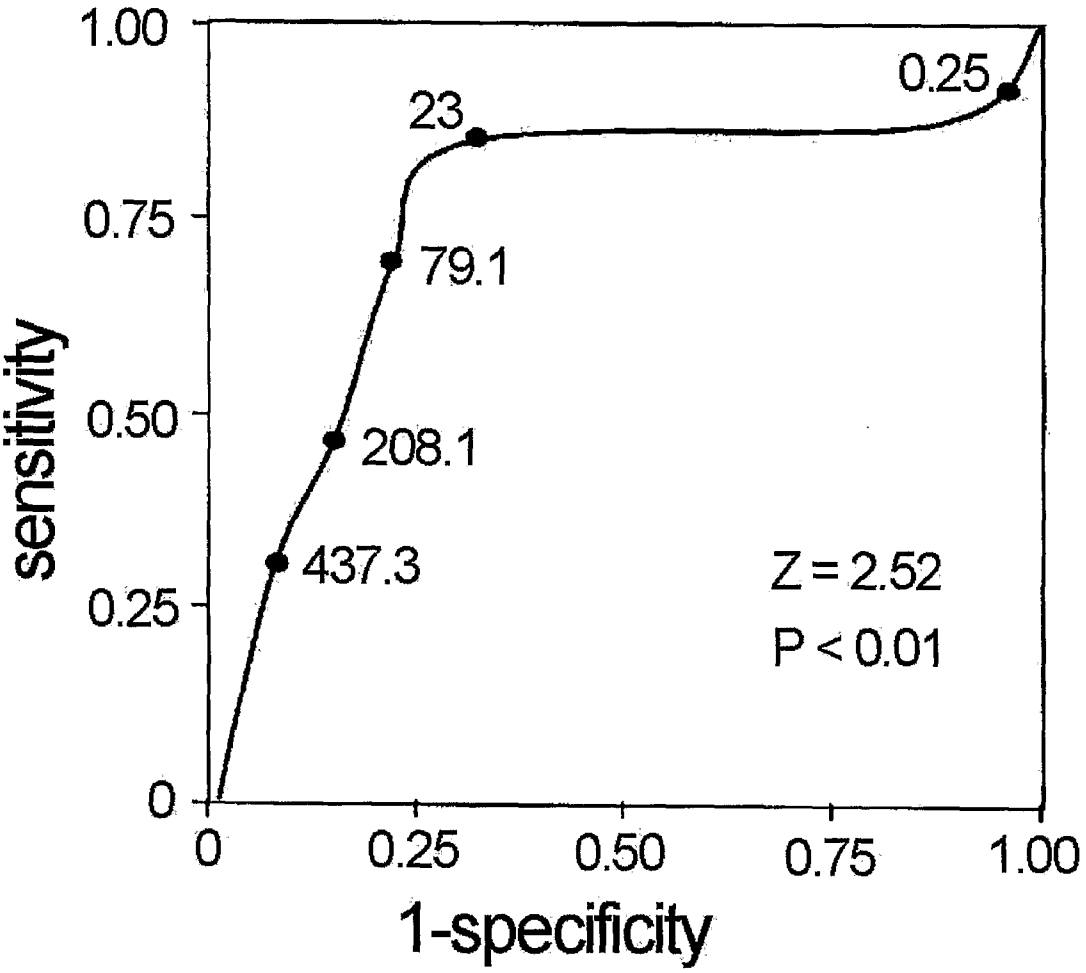

MMP-8 (ng/ml)
3836.8
1535.9
P<0.01
500
400
300
200
100
0
(-) cerebral palsy
(+) cerebral palsy

FIG. 6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0.75
0.50
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0
sensitivity
0
0.25
0.50
0.75
1.00
1-specificity
0.25
23
79.1
208.1
437.3
Z = 2.52
P < 0.01

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/01306

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 G01N 33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC07 G01N, A61K

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched
Korean Patents and applications for inventions since 1975, Korean Utility models and applications for Utility models since 1975
Japanese Utility models and application for Utility models since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
MEDLINE, NPS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WATARI M, WATARI H, DISANTO ME, CHACKO S, SHI GP, STRAUSS JF 3rd.,"Pro-inflammatory cytokines induce expression of matrix-metabolizing enzymes in human cervical smooth muscle cells." In : The American journal of Pathology, American Association of Pathologists and Bacteriologists,1999 Jun, vol.154, no.6, p.1755-62	1-17
A	TU FF, GOLDENBERG RL, TAMURA T, DREWS M, ZUCKER SJ, VOSS HF,"Prenatal plasma matrix metalloproteinase-9 levels to predict spontaneous preterm birth" In: Obstetrics and gynecology, Elsevier North Holland, 1998 Sep, vol.92, no.3, p.446-9	1-17
A	MORRISON JJ, CLARK IM, POWELL EK, CAWSTON TE, HACKETT GA, SMITH SK, "Tissue collagenase:serum levels during pregnancy and parturition" In: European Journal of Obstetrics, Gynecology and reproductive biology, Elsevier Science Pub.Co., 1994 Mar, vol.54, no.1, p.71-5	1-17
A	US 5545616 A (GENETECH, Inc.) 13 August 1996 See the whole document	1-17
A	US 5597700 A (California Research, LLC) 28 January 1997 See the whole document	1-17

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 NOVEMBER 2001 (19.11.2001)	21 NOVEMBER 2001 (21.11.2001)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea	MIN, Man Ho
Facsimile No. 82-42-472-7140	Telephone No. 82-42-481-5578

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/01306

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-8
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-8 are directed to a method for the diagnosis, the search has been carried out and based on the alleged effects of the reagent and kit.

2. [ ] Claims Nos.:
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. [ ] As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR01/01306

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5597700 A	28.01.1997	AU 2416295 B	10.06.1999
		EP 755515 A	29.11.1997
		DE 69513160 C	29.01.1997
US 5545616 A	13.08.1996	NONE	

专利名称(译)	用于产前诊断早产，胎儿感染和胎儿损伤的诊断剂，以及含有该诊断剂的诊断试剂盒		
公开(公告)号	EP1336103A1	公开(公告)日	2003-08-20
申请号	EP2001953363	申请日	2001-08-01
[标]申请(专利权)人(译)	首尔大学校产学协力团		
申请(专利权)人(译)	首尔国立大学的产业基础		
当前申请(专利权)人(译)	首尔国立大学的产业基础		
[标]发明人	YOON BO HYUN305 603 SHIN BANPO 8 CHA APT		
发明人	YOON, BO HYUN#305-603 SHIN BANPO 8-CHA APT.		
IPC分类号	C12Q1/37 G01N33/53 G01N21/78 G01N33/543 G01N33/545 G01N33/552 G01N33/558 G01N33/573 G01N33/577 G01N33/68		
CPC分类号	G01N33/689 C12Q1/37 G01N33/54386 G01N33/558 G01N2333/96486 G01N2800/36 G01N2800/368		
优先权	1020000069283 2000-11-21 KR		
其他公开文献	EP1336103A4		
外部链接	Espacenet		

摘要(译)

本发明涉及用于产前诊断早产，胎儿感染和胎儿损伤的方法，以及用于诊断的诊断试剂系统和诊断试剂盒。该方法，诊断试剂系统和试剂盒基于以下发现：当孕妇处于早产，宫内感染和胎儿损伤的风险中时，羊水中的MMP-8水平显着更高。诊断试剂系统和试剂盒可以应用于患者，而不会出现早产或胎膜早破的临床症状。与常规测量胎儿血液细胞因子水平的方法相比，该灵敏度和特异性以及较低的侵袭性具有优势，该诊断试剂系统和试剂盒在产前诊断早产，胎儿感染和胎儿损伤方面非常有用。