

Diagnosis of Premature Rupture of Membranes with an Improved α -Fetoprotein Monoclonal Antibody Kit

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We developed a new kit for detecting α -fetoprotein (AFP) in leaked amniotic fluid (Eur J Obstet Gynecol Reprod Biol 1995;58:67-72). Later, we developed an improved AFP kit utilizing the same AFP monoclonal antibody. We compared this improved AFP test with the nitrazine test for 137 patients. The nitrazine test correctly diagnosed 62.1% of the cases, but the improved AFP kit diagnosed 98.0% for <37 weeks of gestation ($P < 0.001$). The nitrazine test showed a specificity of 58.3%, whereas the AFP kit showed a 100% rate for detecting ≥ 37 weeks of gestation ($P < 0.01$). The reaction time with the AFP kit is 90 s. This study has confirmed a high clinical efficacy of the improved AFP test kit as a method of diagnosis of premature rupture of fetal membranes.

Indexing Terms: fetal status/amniotic fluid/immunoassay

Premature rupture of fetal membranes (PROM), a fairly common complication of pregnancy, can increase perinatal morbidity and mortality as well as maternal complications.⁴ Conventional diagnostic methods for PROM often produce false-positive and false negative results. Even in late gestation, and using a combination of several conventional diagnostic methods, a correct diagnostic rate of only 93% has been reported for PROM diagnosis (1).

Amniotic fluid contains a high concentration of α -fetoprotein (AFP), especially in preterm pregnancy, and lower or undetectable amounts are present in maternal blood, urine, vaginal fluid, and seminal fluid (2-4). In 1983, Huber et al. (5) found that the concentration of AFP in vaginal fluid increased after PROM, and they attempted to use this in PROM diagnosis. In 1987, Rochelson et al. (6) introduced a colorimetric monoclonal antibody assay method with high sensitivity and specificity, which could be used in PROM diagnosis to measure the AFP concentration in vaginal fluid.

We developed a new anti-AFP monoclonal antibody kit that is easier to use for a bedside test and produces results more quickly than previous methods (7). Now, we have developed an improved AFP monoclonal anti-

body kit, utilizing the same AFP monoclonal antibody (hereafter, called the improved AFP kit). The improvements of the AFP kit are: (a) direct collection of the specimen (leaking amniotic fluid) with a polyester swab developed by us instead of with a syringe; (b) altering the direction of specimen flow in the reaction card from horizontal to vertical; (c) inclusion of a control spot (equivalent to the cutoff concentration) adjacent to the evaluation test spot portion to facilitate comparison; and (d) the reaction time shortened to 90 s.

Here we report the results of our investigation of the basic performance and clinical usefulness of this improved AFP kit in the diagnosis of PROM.

Materials and Methods

Principle of Improved AFP Kit

The specimen addition part of the reaction card is impregnated with lyophilized chromogen-labeled (blue) anti-human AFP monoclonal antibody, and the reaction membrane contains two spots of immobilized antibodies: The control spot is anti-mouse IgG antibody; the test spot is another anti-human AFP monoclonal antibody, which differs in antigen recognition site from the chromogen-labeled antibody. The chromogen-labeled anti-AFP monoclonal antibody is solubilized by the sample solution and migrates onto the reaction membrane placed below, reacting with each of the immobilized antibodies. If the sample contains AFP exceeding the detection limit, the test spot turns blue because of formation of a "sandwich" immune complex: immobilized anti-human AFP monoclonal antibody/AFP/chromogen-labeled anti-human AFP monoclonal antibody. Meanwhile, the control spot binds the chromogen-labeled mouse anti-human AFP monoclonal antibody directly and exhibits coloration with an intensity equivalent to that obtained when reacting with AFP at its cutoff concentration.

Procedures for Improved AFP Kit

Collection of specimen. Bring the swab portion of the specimen collector in contact with the sampling site (cervix, posterior fornix vaginae). Wait 15 s to soak up a sufficient volume of specimen. Remove excessive specimen by sliding the outer tube of the specimen collector toward the tip. Place the swab portion in the specimen diluent container, which holds 700 μ L of 10 g/L bovine serum albumin in phosphate-buffered saline, and cover with the screw cap. Vigorously agitate the container for 10 s to dissolve the specimen collected

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⁴ Nonstandard abbreviations: PROM, premature rupture of fetal membranes; AFP, α -fetoprotein; PRL, prolactin; hPL, human placental lactogen.

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in the swab. This dilution process dilutes the specimen ~41.5-fold.

Assay. Add 450 μL of diluted specimen to the specimen addition part of the reaction card by using the attached pipette. After the diluted specimen has been completely absorbed, immediately drip 10 drops of the rinsing solution onto the addition part. When the rinsing solution has been completely absorbed, turn the card upside down and visually judge the color development of the control and test spots.

If the coloration of the test spot is equivalent to or darker than the coloration of the control spot, the evaluation is "positive"; if the coloration of the test spot is lighter than the coloration of the control spot or if no coloration is detected, the evaluation is "negative"; if no coloration occurs in the control spot, the evaluation is "invalid"; a retest is required.

Subjects

Subjects were 137 patients between the 11th and 40th weeks of gestational age (47 cases of PROM and 90 cases of no PROM) evaluated at the Hokkaido University Hospital from October 1993 through May 1994. The diagnosis of PROM was determined by daily speculum examination of vaginal fluid leakage until 72 h after admission and findings of oligohydramnios on ultrasound. The diagnosis of no PROM was determined by daily inquiry as to the cessation of vaginal fluid loss, findings by speculum examination of the absence of leaking and pooling until 72 h after admission, and no findings of oligohydramnios on ultrasound.

All patients, after their consent, underwent a sterile speculum examination and nitrazine test together with the improved AFP test. Vaginal fluid or cervical secretion samples were used for testing. The nitrazine test was performed in collected samples. The color was compared with a color chart; $\text{pH} \geq 6.5$ was considered positive. To compare the concentration of AFP of the specimen with the results of the improved AFP kit, we stored the samples frozen below -20°C , and measured the concentration by enzyme immunoassay.

Based on our previous investigation (7), the cutoff AFP concentrations of the cervical secretion and the vaginal fluid was set at 125 $\mu\text{g/L}$.

We also investigated the performance of the specimen collector and the reproducibility of the test for the detection sensitivity, cross-reaction, and influence of contaminated substance on reaction in the improved AFP kit.

For statistical analysis, we used the χ^2 test with Yates' continuity correction. $P < 0.05$ was considered significant.

Results

Specimen Collector

Use of outer tube. We compared the amount of specimen collectable from normal pregnant women with and without the use of the outer tube; using the outer tube reduced the CV from 42.1% to 28.7% (Fig. 1).

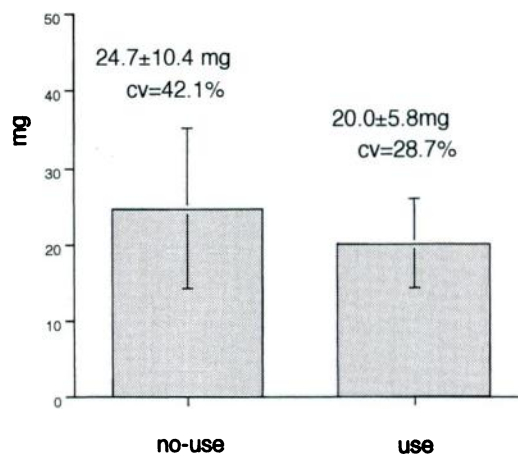


Fig. 1. Effect of the outer tube on the specimen collector on the precision of collection.

Amount collectable with specimen collector. To examine between-collector variation in the amount of collected specimens, three obstetricians gathered 51 specimens of cervical secretion from normal pregnant women. The mean \pm SD mass of collected specimen was 17.3 ± 7.0 mg; the between-collector CV was 12.9%, very low for this kind of procedure.

AFP recovery. Solution and extraction of specimen from the specimen collector into the specimen diluent was performed by agitating the specimen collector swab in the specimen diluent before extraction. The AFP recovery rate after this procedure was confirmed to be 91.9%, 95.2%, and 97.8% by five examinations of each of three different specimens.

Subsequently, to determine the influence of freezing and thawing the specimen, we examined AFP recovery rate for amniotic fluid collected with a specimen collector after subjection to a freeze-thaw cycle. The AFP recovery rate was 100.6% ($n = 6$) when the specimen on the specimen collector was immersed in the diluent and stored frozen and AFP was extracted by agitation after thawing, and 106.8% ($n = 6$) when the specimen was immersed in the diluent, AFP was extracted by agitation, and the extract was then frozen.

Kit Evaluation

The reproducibility of the improved AFP kit was ascertained by the constant negative and positive results that were determined 10 times for different concentrations of purified AFP (0, 1.5, 3, 5, 10, and 50 $\mu\text{g/L}$). These concentrations of purified AFP are equivalent to AFP concentrations of 0, 62.5, 125, 210, 415, and 2075 $\mu\text{g/L}$ in the original cervical-vaginal fluid, respectively. The blue color formation was stable at an AFP concentration of ≥ 3 $\mu\text{g/L}$ by repeated examinations (Fig. 2). No cross-reactions were observed with prolactin (PRL) (5000 $\mu\text{g/L}$), human placental lactogen (hPL) (10 000 $\mu\text{g/L}$), human chorionic gonadotropin (10 000 IU/L), luteinizing hormone (1000 IU/L), and follicle-stimulating hormone (1000 IU/L), respectively. Contamination with glucose (10 g/L), albumin (10 g/L), hemoglobin (800 mg/L), or bilirubin (100 mg/L) did not

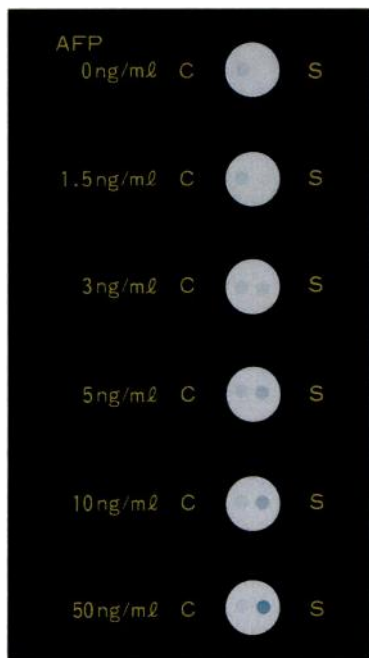


Fig. 2. Coloration of the AFP calibrator solution.

Control spots (C) contain reactant equivalent to 125 $\mu\text{g/L}$ AFP in vaginal fluid. The test spots (S) show the intensity of color development at the indicated concentrations of purified AFP, which correspond to original AFP concentrations in vaginal fluid of (top to bottom) 0, 62.5, 125, 210, 415, and 2075 $\mu\text{g/L}$, respectively.

affect the reactions. The time required for the reaction with this kit was ~ 90 s.

Clinical Study

Of the 47 cases diagnosed with PROM, 46 were positive by the improved AFP kit (Table 1). The actual AFP concentrations of the 47 positive samples were all above the AFP cutoff value of 125 $\mu\text{g/L}$ by enzyme immunoassay. The false negative for this kit involved a case of 39 weeks of gestation in which the original vaginal fluid had an AFP concentration of 42 $\mu\text{g/L}$.

Of the 90 cases diagnosed with no PROM, 88 were negative by the improved AFP kit, which reported undetectable (< 25 $\mu\text{g/L}$) AFP concentrations in 87 cases and 61 $\mu\text{g/L}$ in the remaining case. The two false-positive samples were from a woman at 25 weeks of gestation who had chorioamnionitis (intrauterine infection) and gave a positive result according to the improved AFP test and the nitrazine test (measured AFP concentration, 216 $\mu\text{g/L}$), and a woman at 32 weeks of gestation who had a heavy bloody discharge and demonstrated a positive result by the improved

AFP test and the nitrazine test (measured AFP concentration, 471 $\mu\text{g/L}$).

In summary, the accuracy of diagnosis of the present kit was 97.8%, sensitivity 97.9%, and specificity 97.8%; these values were significant by χ^2 test (Table 1).

For diagnosis efficiency in 103 cases of < 37 weeks of gestation (25 cases of PROM and 78 cases of no PROM), the improved AFP kit was significantly superior (by χ^2 test) to the nitrazine test, giving an accuracy of diagnosis of 98.0%, a specificity of 97.4%, and a false-positive rate of 2.6% (Table 2).

For 34 cases of ≥ 37 weeks of gestation (22 cases of PROM, 12 cases of no PROM), again the improved AFP kit was significantly superior to the nitrazine test at a specificity of 100% and a false-negative rate of 0% by χ^2 test (Table 2).

Discussion

Friedman and McElin (1) report that none of the traditional procedures proved entirely satisfactory on its own but that a combination of any three of a careful history, the nitrazine test, an amniotic fluid crystallization test, or the Nile blue stain produced a diagnostic accuracy of $\sim 93\%$ for the diagnosis of PROM, even at late gestation. The sensitivity of the nitrazine and ferning tests for PROM diagnosis in preterm pregnancies was 77% and 62%, respectively (6).

Obstetricians have long looked forward to having a noninvasive, repeatable, and quick bedside test that provides a much higher efficacy in diagnosing amniotic fluid leakage without having to use the invasive technique of intraamniotic dye injection.

Huber et al. (5) reported an RIA for PROM diagnosis that determined AFP, PRL, and hPL concentrations in vaginal fluid. However, measurement by RIA is not considered practical as a diagnostic method under clinical conditions. In 1983, Rochelson et al. (4), using an anti-AFP antibody, developed a method for PROM diagnosis based on latex agglutination and reported a sensitivity of 93% and a specificity of 94%. However, centrifugation of the extracted solution was necessary in this method. Judgments of the results of the agglutination were often ambiguous, and for many cases an explanation of the diagnostic results was difficult (6). In 1987, Rochelson et al. (6) introduced a colorimetric assay using anti-AFP mouse monoclonal antibody for PROM diagnosis in preterm pregnancies. They re-

Table 2. Accuracy of diagnosis for ruptured membranes by improved AFP kit and by nitrazine test.

	Gestation < 37 weeks (n = 103)		Gestation ≥ 37 weeks (n = 34)	
	AFP kit	Nitrazine	AFP kit	Nitrazine
Accuracy of diagnosis, %	98.0 ^a	62.1	97.1	79.4
Sensitivity, %	100	92	95.5	100
Specificity, %	97.4 ^a	52.6	100 ^b	41.7
False positive, %	2.6 ^a	47.4	0 ^b	58.3
False negative, %	0	8	4.5	0

^{a,b} Significantly different from nitrazine results: ^aP < 0.001 , ^bP < 0.01 .

Table 1. Results of clinical study with the improved AFP kit (n = 137).

	AFP kit (n = 137)		Total
	PROM	No PROM	
Positive	46	2	48
Negative	1	88	89
Total	47	90	137

Accuracy of diagnosis, 97.8%; sensitivity, 97.9%; specificity, 97.8%; P < 0.001 .

ported a sensitivity of 98%, which they claimed made this method more reliable than the nitrazine or ferning method. However, in 1990, Garite and Gocke (8) reported that, using the same colorimetric monoclonal antibody assay, their results showed only a specificity value higher than in the nitrazine test, whereas neither the sensitivity nor the specificity values were better than in the ferning method. They also reported that 15 min was necessary for testing.

We developed a kit utilizing anti-AFP monoclonal antibody (AFP kit) for a rapid (~3 min) and concise test of PROM and previously reported its usefulness (7). With this AFP kit, the specimen was directly collected from the site of sampling with a syringe. However, because the cervical secretion to be collected was frequently highly viscous, sample collection was hindered. Consequently, we devised a specimen collector with an outer tube that allows collection of a highly precise amount of specimen. The present study confirms that, by the use of this specimen collector, easy collection of the correct amount of specimen is feasible. The previous kit used the appearance of a blue band as an indicator for evaluation with the AFP kit; the present improved AFP kit includes a control spot exhibiting coloration with an intensity equivalent to that at the cutoff concentration (125 $\mu\text{g/L}$), placed in the vicinity of the test spot, which facilitates comparison and allows for a more definite evaluation.

When diagnosing PROM with the present improved AFP kit, the accuracy of diagnosis was 97.8%, sensitivity 97.9%, and specificity 97.8%, resulting in significant ($P < 0.001$) diagnostic superiority. In addition, in the clinically important patients of < 37 weeks of gestation, the improved AFP kit, in contrast to the nitrazine test, yielded a significantly ($P < 0.001$) superior accuracy of diagnosis of 98.0% and a specificity of 97.4%. Further, in cases with ≥ 37 weeks of gestation, the improved

AFP kit, in comparison with the nitrazine test, showed a specificity of 100%, confirming diagnostic superiority ($P < 0.01$).

The time required for the reaction with the improved AFP kit is ~90 s, indicating that it is a rapid and concise technique compared with the previous AFP kit; also, visual evaluation with the present kit is clearer. We thus consider this method suitable for use in repeated tests at the bedside or with outpatients.

Although this clinical study involved only a few cases, it still suggests that the present improved AFP kit is useful in diagnosing PROM, especially during preterm pregnancy.

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