

AmniSure Placental Alpha Microglobulin-1 Rapid Immunoassay versus Standard Diagnostic Methods for Detection of Rupture of Membranes

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ABSTRACT

The purpose of this study was to compare the AmniSure rapid immunoassay with standard methods for diagnosing rupture of fetal membranes. Patients presenting with signs/symptoms of membrane rupture between 15 and 42 weeks of gestation were invited to participate. Standard/control methods were performed to establish a diagnosis and compare it with AmniSure results. AmniSure performance metrics and their 95% confidence intervals were calculated. A total of 203 patients agreed to participate. Discrepancies between the control method and AmniSure were noted in seven cases. In these cases, true positives and negatives were determined by retesting with the control method and AmniSure and by noting sonographic evidence of low amniotic fluid. In the final analysis, the AmniSure diagnostic test demonstrated a sensitivity of 98.9%, specificity of 100%, positive predictive value of 100%, and a negative predictive value of 99.1%. AmniSure is highly accurate in diagnosing fetal membrane rupture.

KEYWORDS: AmniSure ROM test, rupture of membranes, PAMG-1 immunoassay

Premature rupture of the fetal membranes (PROM) occurs in approximately 10% of pregnancies. PROM poses one of the most important therapeutic dilemmas in current obstetric practice.¹ PROM is one of the most common diagnoses associated with premature delivery and neonatal complications requiring admission to a neonatal intensive care unit. The management of the patient with PROM and preterm PROM (PPROM) is expensive and remains an important dilemma as the clinician weighs the risk of prolonging gestation against the risks of serious neonatal consequences, such as infection,² preterm delivery,³ fetal distress, prolapsed cord, abruptio placenta, and infection.⁴ PPRM ac-

counts for 20% to 40% of PROM, and the incidence is doubled in multiple gestations. PPRM is associated with 20% to 50% of premature births, infectious morbidity in the mother and fetus, pulmonary hypoplasia of the fetus, prolapse of the umbilical cord, development of fetal deformities, and postnatal endometritis. These consequences significantly increase fetal and maternal morbidity and mortality.⁵ Given that PPRM is associated with 20% to 50% of premature births, PPRM is also responsible for neonatal problems resulting from prematurity.³

Failure to identify patients with PROM can result in the failure to implement salutary obstetric measures. Conversely, an incorrect diagnosis of membrane rupture

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can lead to inappropriate interventions (such as hospitalization or induction of labor). Therefore, the correct and timely diagnosis of this disorder is of critical importance to the clinician because PROM and PPRM may be associated with serious maternal and neonatal consequences.⁶ Accurate diagnosis of fetal membrane rupture, however, remains a frequent clinical problem in obstetrics. Unfortunately, a noninvasive diagnostic gold standard is not available at this time. Currently available tests are inaccurate and require an intrusive examination. The current diagnostic methods using nitrazine/pH, assessment of pooling, and microscopic fern testing lack reliability and become progressively less accurate with passage of time since membrane rupture. In cases of prolonged PROM, these tests provide no better diagnostic information than that obtained by simple clinical evaluation.⁷

Ferning has been associated with false-positive results in 5 to 30% of patients; the result is described secondary to contamination with fingerprints on a slide or contamination with semen and cervical mucus.⁸⁻¹⁰ False-negative results (5 to 12.9%) may be caused by dry swabs, contamination with blood, and heavy discharge.^{8,11,12} In the nonlaboring group, sensitivity and specificity were 51.4% and 70.8%, respectively.¹³

Nitrazine evaluation is associated with false-positive results (up to 17.4%) secondary to cervicitis, vaginitis (bacterial vaginosis or trichomonas), alkaline urine, blood, semen, and antiseptics.^{8,14} A significant false-negative rate (12.9%) was also observed for Nitrazine.⁸ Sensitivity and specificity have been reported at 90.7% and 77.2%, respectively.¹⁵

The absence of a noninvasive gold standard for the diagnosis of membrane rupture resulted in the appearance of several tests based on alternative biochemical markers. Alpha-fetoprotein, vaginal prolactin, fetal fibronectin, and ActimPROM (an insulin-like growth factor binding protein-1 immunoassay) have all failed to produce the accuracy and noninvasiveness required ideally for a gold standard.¹⁶⁻¹⁹ AmniSure is a rapid strip test that can detect a rupture of the fetal membranes, providing highly accurate and timely PROM and PPRM diagnosis. The AmniSure test kit is a self-contained test system that does not require a speculum examination. It is hypothesized that AmniSure will provide qualitative results that will exceed the combined current diagnostic methods of Nitrazine pH evaluation, ferning, and pooling in sensitivity and specificity, with the potential to serve as the gold standard for diagnosing ruptured membranes.

MATERIAL AND METHODS

Eligibility

Patients were recruited from the Triage Unit of the Sharp Mary Birch Hospital for Women (San Diego,

CA) and the Summit Medical Center (Oakland, CA). Pregnant women between 15.0 to 42.0 weeks gestation presenting with signs and/or symptoms of membrane rupture were invited to participate in the study. Patients with active vaginal bleeding from any source and/or known placenta previa were excluded from the study. The study was approved by the Institutional Review Boards at both hospitals. Informed consent was obtained from all subjects.

Study Procedures

Participants who provided consent had both the standard clinical examination and the AmniSure test for PROM performed. The examinations were performed by different procedure-competent clinicians blinded to each other's results. The diagnosis of membrane rupture (control method) required the coinciding positive results of at least two of the following procedures: visual pooling of amniotic fluid, alkaline pH determination (Nitrazine test) of vaginal secretions, and microscopic evidence of ferning. A sterile speculum examination was performed. A sterile Dacron swab was used to collect fluid from the posterior vaginal fornix for the Nitrazine and fern testing. The presence or absence of pooling was visually assessed. Then the AmniSure placental alpha microglobulin-1 (PAMG-1) assay was performed by the second examiner. A Dacron swab was passed along the distal vaginal sidewall. The swab was left in place for 20 to 30 seconds to ensure swab saturation. The swab was then agitated in the AmniSure diluent vial for 1 minute. The diluent-saturated swab was then applied to the AmniSure slide until one or two lines could be seen in the control and test windows. The slide indicated a negative or positive result within 5 to 10 minutes. (The latest version of AmniSure test simplifies this procedure by using a test strip that is dipped directly into the diluent vial, instead of a slide).

After the patient delivered, the clinical record was reviewed to assess whether the patient had PROM or PPRM. The study data were collected, analyzed, and stored by study personnel in a fashion to ensure patient anonymity and confidentiality. The AmniSure PAMG-1 specimens were individually assessed for sensitivity, specificity, positive and negative predictive values, and false-positive and false-negative rates for PROM and PPRM. Discrepancies between the AmniSure and the control method were addressed by detailed review of the patient's clinical course by the local investigator.

Data Analysis

Sensitivity, specificity, and positive and negative predictive values were calculated. Cases with discrepant AmniSure and control test results were analyzed

Table 1 Uncorrected Summary of Patients Tested with AmniSure and Control Methods

Control Test Results				
AmniSure		+	–	Total
Test	+	86	4	90
Results	–	3	110	113
Total		89	114	203

separately. Two datasets were created: uncorrected and corrected results. The uncorrected results were compiled assuming that AmniSure gave the wrong result in cases when discrepancy was observed between AmniSure and the control method (Table 1). The corrected data results were collected after case evaluation. This dataset incorporated resolution of the discrepancy (Table 2).

RESULTS

A total of 203 consenting patients were evaluated. A total of 89 (43.8%) were diagnosed with ruptured membranes using the control method. One hundred fourteen patients (56.2%) were diagnosed as not having ruptured membranes. In seven cases, the control method and AmniSure produced discrepant results. Of these seven cases, four were initially designated as false-positive AmniSure results (1.9% of total patient population). In three of the seven cases, AmniSure was initially designated as false negative (1.4% of total patient sample). Each of these seven discrepant cases was followed up to verify the true diagnosis. The true-positive diagnosis was verified by either retesting with the AmniSure and control methods, or by sonographic evidence of low fluid (i.e., oligohydramnios). True-negative diagnosis was verified by retesting with the control method and with AmniSure. On final analysis, as it is reflected in the corrected dataset (Table 2), AmniSure provided more accurate diagnosis than the control method in all but one case. In this particular case, where AmniSure provided a false-negative result, the investigators suspected a defective test kit. Given that there was no way to verify this suspicion, AmniSure was recorded in the corrected summary of patients' diagnosis as a false negative. The remaining six discrepant cases were resolved in favor of AmniSure producing the final performance metrics

Table 2 Corrected Summary of Patients Tested with AmniSure and Control Methods

Control Test Results				
AmniSure		+	–	Total
Test	+	90	0	90
Results	–	1	112	113
Total		91	112	203

Table 3 AmniSure Performance Metrics

Metric	Derivation (all data)		Value
Sensitivity	TP/(TP + FN)	90/(90 + 1)	98.9%
Specificity	TN/(TN + FP)	112/(0 + 112)	100.0%
PPV	TP/(TP + FP)	90/(90 + 0)	100.0%
NPV	TN/(TN + FN)	112/(112 + 1)	99.1%

PPV, positive predictive value; NPV, negative predictive value; TP, true positives; FP, false positives; TN, true negatives; FN, false negatives.

(Table 3). In four cases, repeat examination concluded that the control method produced false-negative results, whereas AmniSure produced true-positive results.

DISCUSSION

In 1975, Dr. D. Petrunin described the PAMG-1 protein isolated from amniotic fluid.²⁰ He obtained antibodies against the protein and used immunochemical methods to measure the contents of the protein in amniotic fluid at different stages of pregnancy. He was the first to isolate and define a protein marker of amniotic fluid and to measure its concentration in the blood and different organs of the fetus and adult.

PAMG-1 is a 34-kd protein.²¹ It was selected as a marker of fetal membrane rupture due to its unique characteristics (i.e., high concentration in the amniotic fluid, low level in blood, and extremely low background level in cervicovaginal secretions with intact fetal membranes). To minimize the frequency of false results, two monoclonal antibodies have been selected to set the sensitivity threshold of AmniSure at the optimal low level. This level allows the detection of the extremely small quantities of amniotic fluid in vaginal secretions (0.0025 to 0.00025 mL [2.5 to 0.25 μ L] of amniotic fluid per 1 mL of vaginal secretion). Background concentration of PAMG-1 measured by this combination of monoclonal antibodies is 0.05 to 0.2 ng/mL of vaginal secretion. When vaginitis or an admixture of blood or serum is present, the background level of PAMG-1 can rarely reach the maximum of approximately 3 ng/mL. PAMG-1 concentration in amniotic fluid ranges from 2,000 to 25,000 ng/mL. When fetal membranes are ruptured, the PAMG-1 level in vaginal secretions increases significantly. By having its sensitivity threshold set at 5 ng/mL, AmniSure minimizes the probability of false-positive or false-negative results.

The presence of increased levels of protein PAMG-1 in the vaginal secretions is highly predictive of ruptured fetal membranes. The AmniSure PAMG-1 immunoassay provides qualitative results that exceed in timeliness, accuracy, sensitivity, specificity, and reliability the currently available methods. AmniSure does not require the speculum examination used routinely for evaluation for ROM, and can serve as one test that

covers the entire spectrum of diagnostic necessity—from simple cases where confirmatory diagnosis is needed to the most difficult cases where no visible leakage of amniotic fluid is evident or detectable by standard methods (subclinical rupture).

In summary, AmniSure is a rapid, bedside strip test that can detect rupture of fetal membranes with a high degree of predictive accuracy (Table 3). Additional cohort studies are indicated.

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NOTES

Clinical Studies were conducted at Sharp Mary Birch Hospital for Women, San Diego, CA, and Summit Medical Center, Oakland, CA. Clinical studies were unfunded.

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