

AmniSure® ROM (Rupture Of [fetal] Membranes) Test

DIRECTIONS FOR *IN VITRO* DIAGNOSTIC USE

INTENDED USE

The AmniSure® ROM (Rupture Of [fetal] Membranes) Test is a rapid, non-instrumented qualitative immunochromatographic test for the *in vitro* detection of amniotic fluid in vaginal discharge of pregnant women. The test is for use by health care professionals to aid in the detection of fetal membranes rupture in pregnant women with a suspicion of such a rupture.¹

SUMMARY AND EXPLANATION OF THE TEST

Premature rupture of the fetal membranes (PROM) occurs in about 10% of pregnancies. Up to date, PROM poses one of the most important therapeutic dilemmas in current obstetric practice.¹ PROM is perhaps the single most common diagnosis associated with premature delivery and neonatal complications requiring admission to a neonatal intensive care unit. The management of the patient with PROM and PPRM (pre-term PROM) is expensive and remains an important perinatal dilemma as the clinician attempts to balance the risk of prolonging gestation against the risks of infection.¹

The risks of PROM at term are related to serious neonatal consequences, such as pre-term delivery,² fetal distress, prolapsed cord, abruptio placenta and infection.³ PPRM refers to PROM that occurs before 37 weeks' gestation. It accounts 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, postnatal endometritis. All these

consequences significantly increase fetal and maternal morbidity and mortality.⁴ The latency period in PPRM is inversely related to the gestational age thereby increasing the risks of consequences in very premature infants and their mothers. Since PPRM is associated with 20% to 50% of premature births, PPRM is also responsible for the neonatal problems resulting from prematurity.⁵

Failure to identify patients with PROM can result in the failure to implement salutary obstetric measures (for example, Neonatal Sepsis occurs when PROM is diagnosed more than 32 hours after its actual incidence). Conversely, the false diagnosis of membranes rupture can lead to inappropriate interventions (e.g., hospitalization or induction of labor). **Therefore the correct and timely diagnosis of this disorder is of crucial importance for the clinician.**⁶ Accurate diagnosis of fetal membranes ruptures, however, remains a frequent clinical problem in obstetrics.⁷ The timely diagnosis of this disorder is crucial since the PROM is associated with serious neonatal and maternal consequences. Unfortunately, there is no "gold standard" available for the diagnosis of membranes rupture in the clinical practice at this time. Most currently available tests are inaccurate and in some degree invasive. Currently prevalent Nitrazine and Ferning tests are highly unreliable, becoming progressively inaccurate when more than one hour has elapsed since the rupture of the membranes, and becoming completely unreliable after 24 hours. It is concluded that in cases of prolonged PROM these tests provide no better diagnostic

information than that obtained by simple clinical evaluation.⁸

AmniSure® solves these problems. It is a rapid strip test that can detect a rupture of the fetal membranes, providing highly accurate and timely PROM diagnosis. Consequently, measures can be taken in a timely manner to prevent complications (prophylactic use of antibiotics, tocolytic drugs or corticosteroids, labor induction etc).

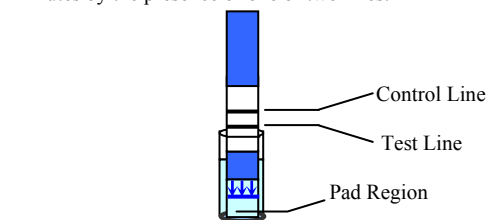
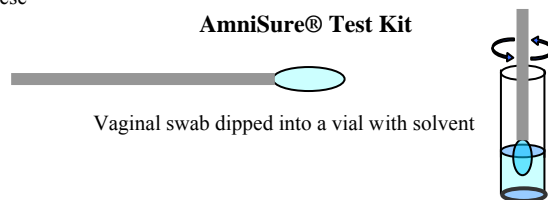
TEST BENEFITS

The AmniSure test kit is a self-contained test system providing qualitative results that exceed in timeliness, accuracy, sensitivity, specificity and reliability the currently available methods. AmniSure is one test that covers the entire spectrum of diagnostic necessity – from simple cases where confirmatory diagnosis is needed to most difficult cases where no visible leakage of amniotic fluid is evident (so-called sub-clinical ruptures). The test does not require speculum examination that is used routinely today for ROM diagnosis.

PRINCIPLE OF THE TEST

Sample of amniotic fluid (taken by vaginal swab) is placed into a vial with solvent. Solvent extracts the sample from the swab for one minute, after which the swab is disposed. AmniSure® Test Strip (a lateral flow device) is then dipped into the vial. The sample substance flows from the Pad Region of the Test Strip to the Test Region. The test result is read visually over next 5-10 minutes by the presence of one or two lines.

Sample of vaginal secretion is taken by vaginal swab



Test Strip is dipped into the vial

The AmniSure ROM Test uses the principles of immunochromatography to detect human PAMG-1 (placental α 1-microglobulin) protein present in amniotic fluid of pregnant women. Placental Microglobulin was selected as a marker of fetal membranes rupture due to its unique characteristics, i.e. its high level in the amniotic fluid, low level in blood and extremely low background level (50-220 picogram/ml) in cervico-vaginal discharge when the fetal membranes are intact.

The test employs highly sensitive monoclonal antibodies that detect even a minimum amount of the protein, which is present in cervico-vaginal discharge after the rupture of the 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 discharge (0.05-0.005 of a drop). Background concentration of PAMG-1 that uses this combination of monoclonal antibodies is around 50-220 picogram (i.e. 0.05-0.22 ng) per 1 ml of vaginal secretion. The sensitivity cut-off of our test is 5-7 ng/ml, i.e. at least 20 times higher than the background concentration. This gap allowed increasing the accuracy of our test to over 99%, since it effectively eliminated false positive and false negative results.¹

During the test procedure, Placental Microglobulin from the sample sequentially binds to a monoclonal antibody (A-Mab) conjugated with the label particles, and then to another monoclonal antibody (B-Mab), immobilized on an insoluble carrier. When A-Mabs come in contact with PAMG-1 on the Pad Region, they "catch" PAMG-1 and transport it to the test region. Test region of the test strip has B-Mabs immobilized on it. B-Mabs "meet" PAMG-1 bound to A-Mabs flowing up from the Pad Region. This "meeting" immobilizes the system of PAMG-1/A-Mabs, resulting in a brown/yellow Test Line that becomes visible in the test region. This Line is produced by dye

gold attached to A-antibodies and indicates a rupture of fetal membranes. The second Control Line is designed to provide indication that the Test is functioning well. This line appears when rabbit anti-mouse IgG antibody catches the mouse A-antibody with dye gold. Dye gold gives the resulting line its color.

REAGENTS AND COMPONENTS

Materials Provided: 1. Directions for use 2. AmniSure Test Strip (lateral flow device) in foil pouch with desiccant. Each Test Strip contains: a) A-Monoclonal antibody (produced by mouse hybridoma) b) Immobilized B-Monoclonal antibody (produced by a different mouse hybridoma) c) Colloidal gold particles linked to A-Monoclonal antibody d) Mouse labeled IgG e) Rabbit anti-mouse IgG antibody 3. Sterile Polyester vaginal swab 4. Plastic vial with water solvent. Solvent solution contains: a) 0.9% NaCl b) 0.1% Triton X100 c) 0.01% NaN₃

PRECAUTIONS AND WARNINGS

*AmniSure test kit is for *in vitro* diagnostic use only. Do not take any part of this internally. *Read and follow exactly the directions for use. Failure to do so may result in inaccurate results. *Safety precautions should be observed when collecting, handling, and disposing of test samples. *Do not use damaged components of the test. *Used test kits are biohazardous. *Take proper precautions when handling/discarding used test kits. *Do not use after the "Use By" date, which is printed on the foil pouch and on the box labeling. *Do not reuse the test kit components. *Do not bend or fold the Test Strip or the aluminum foil pouch with the Test Strip in it.

STORAGE AND STABILITY

*Store the kit in a dry place at 4 to 24°C (40 to 75°F). DO

NOT FREEZE. *When stored in the foil pouch at the

recommended temperature, the test is stable until the "Expiration" date on the box. *AmniSure test should be used within six (6) hours after removing from foil pouch.

TEST PROCEDURE

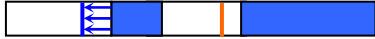
1. Take the solvent vial by its cap and shake well to make sure all liquid in the vial has dropped to the bottom. Open the solvent vial and put it in a vertical position.
2. To collect a sample from the surface of the vagina use the sterile Polyester swab provided. Remove the sterile swab from its package following instructions on the package. The Polyester tip should not touch anything, prior to its insertion into vagina. Hold the swab in the middle of the stick and, while a patient is lying flat on her back carefully insert the Polyester tip of the swab into the vagina until the fingers contact the skin (no more than 5-7 cm deep). Withdraw the swab from the vagina **after 1 minute**.
3. Place the Polyester tip into the vial and rinse the swab in the solvent by rotating for one minute.
4. Remove and dispose of the swab.
5. Tear open the foil pouch at the tear notches and remove the AmniSure test strip.
6. Dip the white end of the Test Strip (marked with arrows) into the vial with solvent. Strong leakage of amniotic fluid will make the results visible early (after 5 minutes), while a very small leak will take the full 10 minutes.
7. Remove the Test Strip if two stripes are clearly visible in the vial (no earlier than 5 minutes) or after 10 minutes sharp. Read the results by placing the test on a clean, dry, flat surface. Do not read or interpret the results after 15 minutes have passed since dipping the Test Strip into the vial.

2.



INTERPRETATION OF RESULTS

One line, NO MEMBRANES RUPTURE:



** The darkness of the stripes may vary. The Test is valid even if the stripes are faint or uneven. Do not try to interpret the test result based on the darkness of the stripes.

QUALITY CONTROL

Each AmniSure test has built-in reagent and procedural controls to make sure it is working properly. The appearance of one or two lines in the test results area verifies the integrity of the test procedure. If only a Control Line is visible the test result is negative. If both Control Line and Test Line are visible, the test result is positive. If no lines are visible, the test result is invalid.

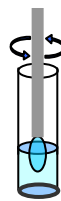
LIMITS OF THE TEST

- The AmniSure Fetal Membranes Rupture Test is for the in vitro detection of human amniotic fluid PAMG-1 protein in vaginal secretion of pregnant woman. The test should be used to evaluate patients with suspicion of and/or clinical signs/symptoms suggestive of fetal membranes rupture
- You must follow all directions carefully to get an accurate reading of the results
- Each test is a single use disposable unit and cannot be reused
- The AmniSure Fetal Membranes Rupture Test results are qualitative. No quantitative interpretation should be made based on the test results
- When there is significant discharge of blood (Polyester end of swab is red), the test can malfunction and is not recommended. In cases of only trace amounts of blood (Polyester swab is pinkish), the test still functions properly.
- In very rare cases when a sample is taken 12 hours or later after a rupture, a false negative result may occur.
- AmniSure® should not be used earlier than 6 hours after the removal of any disinfectant solutions or medicines from the vagina.

EXPECTED VALUES

Leakage of amniotic fluid is indicative of the fetal membranes rupture in all women. Studies of placental α -1 microglobulin protein (PAMG-1) have established it as a marker of amniotic fluid.^{9,10} Concentration of PAMG-1 in cervical and vaginal discharge of pregnant women without complications in pregnancy was measured and is ranged from 0.05 to 0.22 ng/ml. When vaginitis or non-significant admixture of blood serum is present, the background level of PAMG-1 can reach the maximum of 3 ng/ml. PAMG-1 concentrations in the amniotic fluid fall into 2,000-25,000 ng/ml range. Clinically significant

3.



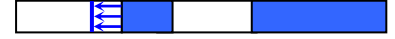
Two lines, THERE IS A RUPTURE:



6.



No line, TEST IS INVALID, take another test:



leakage of amniotic fluid increases PAMG-1 concentration in cervico-vaginal discharge by a factor of thousands. The sensitivity threshold of the AmniSure Test is set by a factor of 20 above the background level of PAMG-1 (AmniSure detects 5 ng/ml of PAMG-1). This eliminated the dependence of test results on possible variations of expected values (levels of PAMG-1) in any given population.

PERFORMANCE CHARACTERISTICS

The clinical performance of the AmniSure test was determined at two independent clinical trials. One study involved single clinical site in Moscow, Russia. Another study involved two sites in California, US.

Results of clinical trials¹:

Sensitivity: **99%**. Specificity: **100%**. PPV (Positive Predictive Value): **100%**. NPV (Negative Predictive Value): **99%**.

Parallel to Nitrazine and Ferning tests, admitted women were tested with AmniSure. Subsequently, especially when there was a discrepancy between the results obtained by AmniSure vs. other tests, the patient was placed under careful clinical observation. In all cases when there was a discrepancy between AmniSure-obtained results and those of other tests, subsequent observation of the patient has proved AmniSure as the test that provided correct diagnosis. The procedure described above resulted in two final sets of data, "corrected results" and "not corrected results". The not corrected results were compiled assuming that AmniSure gave wrong result in cases when discrepancy was observed between AmniSure and currently prevalent tests. Given that such discrepancy gave rise to further observation and testing of the patient and that in all cases of discrepancy AmniSure proved to give the correct result, "corrected data" were compiled. Corrected results therefore incorporated the resolution of the discrepancy, reflecting AmniSure's proven superiority to other tests.

Interference Studies

Vaginal infections or urine do not interfere with the results of AmniSure test. Detailed research and analysis showed that PAMG-1 concentration in vaginal exudates during infections never exceeds the level of 3 ng/ml. AmniSure's sensitivity level is 5-7 ng/ml, excluding any interferences resulting from infections. Concentration of

PAMG-1 in sperm was found not to exceed 4 ng/ml. Concentration of sperm PAMG-1 in vaginal secretion is even lower due to four-time dilution effect during testing. Therefore, during the development of AmniSure and during clinical trials, there was no interference of sperm factor in the results. The same is true for urine. Fifteen samples of urine were studied for PAMG-1 concentration in it, using ELISA. Sensitivity of ELISA was 0.5 ng of PAMG-1 per 1 ml of solution. Parallel to that, AmniSure was also used to detect PAMG-1 in urine. Samples have been obtained from pregnant women at 25-40 weeks of pregnancy. Both methods gave negative results: PAMG-1 has not been found.

Cross Reactivity

The specificity of monoclonal antibodies used in AmniSure was tested by studying their cross-reactive binding to proteins: alpha-2-microglobulin of fertility, human chorionic gonadotropin, trophoblastic beta-1-glycoprotein, human placental lactogen, alpha-fetoprotein, human serum albumen, and some IGFBP proteins. Monoclonal antibodies "A" and "B" used in AmniSure were not cross-reactive to other proteins, except that antibody "B" used in the Test Line was found cross-reactive to IGFBP-3 protein in ELISA. It was shown that concentration of IGFBP-3 in vaginal secretion of pregnant women reaches 680 ng/ml, but this concentration does not impact the sensitivity of AmniSure test to PAMG-1.

Stability of Results

AmniSure's test results can be read in 5-10 min after the Test strip is dipped into the vial. Stability tests were conducted where a lot containing one thousand AmniSure kits has been studied. Purified PAMG-1 has been used in concentrations of 10 ng and 5 ng of PAMG-1 per 1 ml of physiologic saline solution.

The duration/stability of results was also measured. After the result became visible (lines appeared in the test region), it remained stable for at least 5 min. This kind of stability is observed when PAMG-1 concentration is very small (5-10 ng/ml). When the concentration is higher the lines remain stable for hours. AmniSure therefore shows 5 min stability of results, when it takes full 10 minutes for the results to appear.

Table 1. Stability Study Results

Concentration of the Purified PAMG-1 per 1 ml of salt solution (ng/ml)	Time elapsed before the result appeared in the test region of AmniSure test
10	~2.5 min
5	~5.5 min

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