

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. AmniSure® detects PAMG-1 protein marker of the amniotic fluid in vaginal discharge. The test is for use by health care professionals (*Rx ONLY) to aid in the detection of ROM in pregnant women reporting signs, symptoms, or complaints suggestive of ROM.

SUMMARY AND EXPLANATION OF THE TEST

The timely and accurate diagnosis of rupture of [fetal] membranes (ROM) is crucial since the ROM may be associated with serious neonatal and maternal consequences. Failure to identify patients with ROM can result in the failure to intervene appropriately.

Sample of vaginal discharge is taken by vaginal swab



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. 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 1ml of vaginal discharge. The sensitivity cut-off of AmniSure® is 5 ng/ml, i.e. at least 20 times higher than the background concentration. This gap allowed increasing the accuracy of AmniSure®.

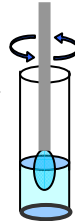
During the test procedure, placental microglobulin from the sample sequentially binds to the monoclonal antibody conjugated with the label particles, and then to another monoclonal antibody, immobilized on an insoluble carrier. When conjugated antibodies come in contact with PAMG-1 on the Pad Region, they "catch" PAMG-1 and transport it to the Test Region. The Test Region of the test strip has antibodies immobilized on it. These antibodies "meet" PAMG-1 bound to conjugated antibodies flowing up from the Pad Region. This "meeting" immobilizes the system of PAMG-1/conjugated antibodies, resulting in a brown/yellow test line that becomes visible in the Test Region. This line is produced by gold dye attached to conjugated antibodies and indicates a Rupture Of [fetal] Membranes. The second control line is designed to indicate that the test is functioning well. This line appears when rabbit anti-mouse IgG antibody "catches" the mouse antibody with gold dye. Gold dye gives the resulting line its color.

Conversely, the false diagnosis of ROM can lead to inappropriate interventions (e.g., hospitalization or induction of labor). Therefore the correct and timely diagnosis of ROM is of crucial importance for the clinician.¹ Accurate diagnosis of [fetal] membranes rupture, however, remains a frequent clinical problem in obstetrics.¹⁻³

Currently available tests have limitations and in some degree are invasive.¹ AmniSure® addresses these problems. It is a rapid non-invasive strip test that can aid in the detection of ROM, providing rapid, easy-to-interpret and timely diagnosis. Consequently, measures can be taken in a timely manner to prevent complications. In clinical trials, one AmniSure® Test correlated with clinical diagnosis obtained through combined usage of three routinely used tests (Nitrazine, Ferning, and Pooling).

AmniSure® Test Kit

Vaginal swab dipped into a vial with solvent



REAGENTS AND COMPONENTS

Materials Provided: 1. Directions for use 2. AmniSure® test strips (lateral flow devices), each in foil pouch with desiccant. Each Test Strip contains: a) Monoclonal antibody (produced by mouse hybridoma) b) Immobilized Monoclonal antibody (produced by a different mouse hybridoma) c) Colloidal gold particles linked to monoclonal antibody d) Mouse IgG labeled by colloidal gold particles e) Rabbit anti-mouse IgG antibody 3. Sterile polyester vaginal swabs 4. Plastic vials with water solvent. Solvent solution contains: a) 0.9% NaCl b) 0.01% Triton X100 c) 0.01% NaN₃

LIMITS OF THE TEST

*The AmniSure® ROM Test is for the *in vitro* detection of human amniotic fluid PAMG-1 protein in vaginal discharge of pregnant women. The test should be used to evaluate patients with 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® ROM Test results are qualitative. No quantitative interpretation should be made based on the test results *

When there is a significant presence of blood on the swab, the test can malfunction and is not recommended.

In cases of only trace amounts of blood on the swab, 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 due to obstruction of the rupture by fetus or resealing of the amniotic sac

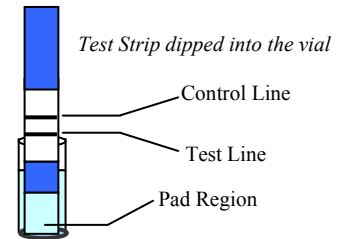
*AmniSure® should not be used earlier than 6 hours after the removal of any disinfectant solutions or medicines from the vagina *Test performance in patients without signs or symptoms of ROM is unknown

*Results should be used in conjunction with other clinical information *Placenta previa, and performing digital exams prior to sample collection can lead to inaccurate test results *Failure to detect membrane rupture does not assure the absence of membrane rupture. *Women may labor spontaneously despite a negative test result *False negative results and delay in the diagnosis of rupture of membranes can increase the risk of chorioamnionitis, oligohydramnios and [fetal] umbilical cord accident. *The performance of the AmniSure® Test has not been established in the

The AmniSure® test kit is a self-contained test system providing qualitative results that are both accurate and do not require collection methods such as speculum examination.

PRINCIPLE OF THE TEST

The test does not require speculum examination that is used routinely today for ROM diagnosis. A sample of amniotic fluid (taken by vaginal swab) is placed into a vial with a solvent. The solvent extracts the sample from the swab for one minute, after which the swab is disposed. The AmniSure® test strip, a lateral flow device, is then dipped into the vial. The sample substance flows from the Pad Region of the strip to the Test Region. The test result is indicated visually by the presence of one or two lines. One line indicates no membranes ruptured; two lines indicate there is a rupture.



presence of the following contaminants: meconium, anti-fungal creams or suppositories, K-Y Jelly, Monistat, Baby Powder (Starch and Talc), Replens, or Baby Oil

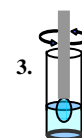
STORAGE AND STABILITY

*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 "Use By" date on the foil pouch. *AmniSure® Test should be used within six (6) hours after removing from foil pouch.

TEST PROCEDURE

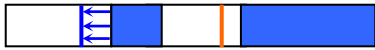
1. Take the solvent vial by its cap and shake well to make sure all liquid in the vial has dropped on the bottom. Open the solvent vial and put it in a vertical position.
2. To collect a sample from the surface of the vagina use the sterile polyester swab provided. Remove the sterile swab from its package following instructions on the package. The polyester tip should not touch anything prior to 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 2-3 inches (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 may make the results visible early (within 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 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:



Two lines, THERE IS A RUPTURE:



No line, TEST IS INVALID, take another test:



** 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.
 *** If only a control line is visible, the test result is negative. If both control and test lines are visible, the test result is positive. If no lines are visible, the test result is invalid.

QUALITY CONTROL

Each AmniSure® Test has built-in reagent and procedural controls to assure accurate reading of the results. The appearance of one or two lines in the test results area verifies the integrity of the test procedure. It is recommended to use external controls and to follow federal, state, and local guidelines for quality control requirements. Freeze-dried PAMG-1 protein is recommended for positive external control and can be purchased through the manufacturer of AmniSure®. Saline solution is recommended for negative external control.

PRECAUTIONS AND WARNINGS

- *AmniSure® is for *in vitro* diagnostic use only. Do not take any part of the test internally.
- A false-negative test may result in an inadequate level of care for newborns less than 37 weeks gestation if device is used in institutions other than those equipped to care for preterm infants (e.g. Level II-III nurseries).
- False negative results can delay the diagnosis of rupture of membranes and can increase the risk of chorioamnionitis, oligohydramnios and fetal umbilical cord accident. Negative results alone may not rule-out membrane rupture.
- The performance of the AmniSure® Test has not been established in the presence of meconium in the amniotic fluid.
- 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.
- Interrupted leakage with minimal residual fluid can lead to false negative result.
- Until the diagnosis of membrane rupture is excluded, avoid digital cervical examination to prevent infection and shorten the latency period.

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.^{4,5} 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 3ng/ml. PAMG-1 concentrations in the amniotic fluid fall into 2,000-25,000 ng/ml range. Clinically significant 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 5-7 ng/ml of PAMG-1).

PERFORMANCE CHARACTERISTICS

The clinical performance of the AmniSure® test was determined by three studies where it was compared to clinical diagnosis provided by a combination of routinely used Nitrazine, Ferning and Pooling tests.^{1,7} The diagnosis was set when two out of three control tests gave identical results (2-out-of-3 method). A total of 432 patients were evaluated at different sites. Of these patients, 108 were from women below 34 weeks gestation; the majority of those 108 (70%) were above 24 weeks. Patient gestational age ranged from 11- 41 weeks. Exclusion criteria included active vaginal bleeding from any source and placenta previa. Statistical analysis is available for these 432 cases. Relative to clinical diagnoses determined by routine clinical tests, AmniSure®'s sensitivity, specificity, and 95% Confidence Intervals (CI) were estimated as follows:

Site	# of Patients	Sensitivity (95% CI)	Specificity (95% CI)
Sharp ¹	203	98.9% (93.2-99.9%)	100.00% (95.9-100%)
Yale/Seoul ⁷	183	98.7% (95.1-99.8%)	87.5% (66.5-96.7%)
Wesley	46	100.00% (83.4-100%)	100.00% (80.8-100%)
Total	432	98.9% (96.6-99.7%)	98.1% (94.1-99.5%)

Pooled Data:

	<34 weeks ^{1,7} (95% CI)	≥34 weeks (95% CI)
Sensitivity	98.3% (90.9-99.9%)	99.07% (96.8-99.8%)
Specificity	95.9% (86.0-99.5%)	99.07% (95.4-99.9%)

Parallel to Nitrazine, Ferning, and Pooling tests, admitted women were tested with AmniSure®.

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 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 discharge 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 used in AmniSure® were not cross-reactive to other proteins, except that antibody 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 discharge 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 if two stripes are clearly visible in the vial or 10 minutes sharp 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. In using the AmniSure®, it is recommended that the results not be read or interpreted after 15 minutes are passed after the Test strip is dipped into the vial.

BIBLIOGRAPY

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