TITLE: AMNISURE ROM (Rupture of [fetal] Membrane Test)  
Performed by the Family Birthing Center

PURPOSE:
The AmnSure ROM test is a rapid, non-instrumented, qualitative test for the detection of amniotic fluid in vaginal secretions of pregnant patients who report signs, symptoms or complaints suggestive of rupture of membranes. Rupture of membranes (pPROM) prior to 37 weeks’ gestation complicates up to 12% of all pregnancies.\(^1\) It uses the principle of immunochromatography to detect human PAMG-1 (placental a-1 microglobulin) protein present in amniotic fluid of pregnant women. Placental Microglobulin was selected as a marker of fetal membrane rupture due to its unique characteristics, i.e. its high level in the amniotic fluid, low level in blood, and extremely low background level in cervico-vaginal secretions when the fetal membranes are intact.

PRINCIPLE OF THE TEST:
The test does not require a speculum exam. A sample of amniotic fluid (taken by vaginal swab) is placed into a vial with solvent. The solvent extracts the sample from the swab for one minute, after which the swab is disposed. The AmnSure test strip, a lateral flow device, is then placed into the vial. The solvent containing antibodies to the PAMG-1 flows from the pad region of the strip to the Test Region. If PAMG-1 is present in the patient sample it will bind with antibodies in the test region producing a second line. The test result is indicated visually over the next 5-10 minutes. One line (Control) indicates no membranes are ruptured. Two lines indicate there is a rupture.

This method is classified by the Clinical Laboratory Improvement Amendments of 1988 (CLIA’88) as a “moderately complex” test procedure (per FDA update 7/19/04). FDA approved the use of this test by Nurses and Midwives, as well as physicians. This test is used for definitive purposes.

AmnSure is now approved for use at any gestational age.

REAGENT AND MATERIALS NEEDED:
- AmnSure ROM Kit in foil pouch with desiccant (FMRT-1-25 for 25 test kit; FMRT-1-10 for 10 test kit)
- Sterile polyester swab (supplied in kit)
- Plastic vial with solvent (supplied in kit; contains 0.9% sodium chloride, 0.01% triton x 100, 0.01 NaN3)
- Timer

STORAGE AND STABILITY:
Storage Requirements:
- Store kits in dry location at room temperature 4-20 °C (40-68°F)
- Kits may be used until printed expiration date.
- Once AmnSure Test Strip is removed from foil pouch, it must be used within 6 hours.
QUALITY CONTROL:

- QC will be performed on each new lot and/or each new shipment received. The log of lot numbers will be kept with the POCT specialist in the central lab.
- QC will be performed if there is suspicion that the product performance is compromised.
- QC will be performed if Amnisure has not been stored according to its labeling specifications.

**Internal Controls**

- AmniSure ROM strip contains built-in reagent and procedural controls to assure accurate reading of the results.
- The appearance of one or two lines in the test area verifies the integrity of the test procedure. The appearance of the control line assures that adequate sample volume was present and that adequate capillary migration (lateral flow) of the sample has occurred.
- It also verifies proper assembly of the test strip by manufacturer.

**External Controls:**

- Prior to patient testing, external positive and negative controls must be run with random kits selected from each new shipment of AmniSure ROM kits to verify performance (i.e. validation or acceptability testing).
- External controls will also be run whenever there is suspicion that product performance is compromised or whenever kits have not been stored according to its labeling instructions.
- The External Controls will be provided by Point of Care Testing, POCT SPECIALIST – LAB.

**Quality Control Procedure:**

- External Positive Control: Fresh human amniotic fluid
- External Negative Control: Saline solution.
- After dissolving amniotic fluid with the saline solution (or distilled water), the obtained solution can be stored under refrigeration at 4-8°C for up to 24 hours. It is preferred, however, to run the QC procedure immediately after preparing a sample of diluted amniotic fluid.

**External Positive Control – Procedure:**

- Take a glass test tube containing 0.1 ml of human amniotic fluid and add 4.9 ml of saline solution. Mix for a few seconds.
- Take 0.25 ml of solution in made in step above and transfer to a small glass tube containing 0.75 ml of saline solution to obtain a 1.0 ml solution with minimal PAMG-1 concentration. Mix.
- Transfer the solution to a vial similar to the vial supplied in the AmniSure test kit.
- Dip the white end of the test strip into the vial with solvent for exactly 10 minutes.
- Remove the test strip after exactly 10 minutes.
- Read results by placing the test strip on a clean, dry, flat surface.
- Do not interpret results after 15 minutes have passed since dipping test strip into vial.

**Negative External Control - Procedure:**

- Using saline solution (or distilled water) for the external negative control, follow steps of the above procedure. Document external QC on QC log.
SAMPLE COLLECTION:
Specimen Requirements:

Patient Preparation:

NOTE: Until the diagnosis of membrane rupture is excluded, avoid digital cervical examination to prevent infection and shorten the latency period.

Identify patient according to patient identification policy.
Position patient flat on back.

Type:
- Collect sample of vaginal secretions using sterile vaginal swab provided in kit.
- Remove swab from packaging using care not to touch anything prior to insertion into vagina.
- Collect sample from surface of vagina, holding swab in the middle of the stick while patient is lying flat on back.
- Carefully insert the polyester tip of the swab into the vagina until fingers contact the skin no more than 2-3 inches (5-7 cm) deep.
- Withdraw the swab after 1 minute.
- Rinse swab after collection in solvent vial for 1 minute, and dispose of as indicated in test procedure.
- Test the patient sample as soon as possible after collection.
- If patient sample is not tested within 30 minutes and sample storage is necessary, tightly close the sample vial and place in ice water for no more than 8 hours.

PROCEDURE:

- Open AmniSure kit, remove and label blue-capped solvent vial with patient information.
- Holding solvent vial by cap, shake well to ensure all liquid in vial is on bottom of vial.
- Open vial and place into rack to keep vertical.
- Place the tip of the polyester swab with patient sample into the labeled, prepared vial and rinse in the solvent by rotating for 1 minute.
- Remove and dispose of the swab according to standard precautions.
- Tear open foil pouch at the tear notches and remove AmniSure test strip.
- Dip white end of strip (marked with arrows) into the correct, labeled vial of solvent.
- Allow strip to remain in vial for 10 minutes, unless 2 lines are clearly visible.
  NOTE: Strong leakage of amniotic fluid will make results visible after 5 minutes, while a small leak may take up to 10 minutes.
- Read the results by placing the strip on a clean, dry flat surface.
- Do not read or interpret results after 15 minutes have passed since placing test strip into vial.
- Positive results can be read as soon as two lines appear in the test region. Negative results will still require a full TEN minutes in order to ensure accurate interpretation.
INTERPRETATION OF RESULTS:

**Reporting Results:** There are 3 possible result interpretations:

- **Only Control Line Present in Test Area:** NO MEMBRANE RUPTURE
- **Control Line and Test Line Present in Test Area:** THERE IS A RUPTURE
- **Control Line Not Present in Test Area:** TEST INVALID, repeat specimen collection and testing

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

**Reporting Format:**

- Document the internal control QC for one patient each day of patient testing.
- All AmniSure patient results must be documented on the patient chart and must be accompanied by the internal QC, date and time of testing and operators initials.

**CLINICAL SIGNIFICANCE:**

Leakage of amniotic fluid is indicative of the fetal membrane rupture in all women. Studies of placental a-1-microglobulin protein (PAMG-1) have established it as a marker of amniotic fluid. Concentration of PAMG-1 in cervical and vaginal secretions of pregnant women without complications in pregnancy was measured and is ranged from 0.05 to 0.22ng/ml. When vaginitis or non-significant admixture of blood is present, the background level of PAMG-1 can reach the maximum level of 3ng/ml. PAMG-1 concentrations in the amniotic fluid fall into the 2,000-25,000 ng/ml range. Clinically significant leakage of amniotic fluid increases PAMG-1 concentration in cervico-vaginal secretions 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).
LIMITATIONS OF TESTING:

- AmniSure test kit is for the in vitro detection of human amniotic fluid PAMG-1 protein in vaginal secretion of pregnant women. The test should be used to evaluate patients with clinical signs/symptoms suggestive of fetal membranes rupture.
- Until the diagnosis of membrane rupture is excluded, avoid digital cervical examination to prevent infection and shorten the latency period.
- Exclusion criteria include active vaginal bleeding from any source and placenta previa.
- Interrupted leakage with minimal residual fluid can lead to false negative result.
- Operators 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 results obtained are qualitative and no quantitative interpretation should be made based on the results.
- The significant presence of blood, collected with the swab, can lead to a false positive result. Insignificant blood admixture does NOT interfere with test results.
- In very rare cases when a sample is taken 12 hours or later after a rupture, a false negative result may occur due to the obstruction of 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 always be used in conjunction with other clinical information.
- Bleeding, placenta previa and performing digital exams prior to sample collection can lead to inaccurate test results.
- Women may labor spontaneously despite a negative test result.

INTERFERING SUBSTANCES:

- Vaginal infections or urine do not interfere with the results of the AmniSure test.
- The performance of AmniSure has not been established in the presence of the following contaminants: meconium, anti-fungal creams or suppositories, K-Y Jelly, Baby Powder (Starch and Talc), Replens, and Baby Oil.
- Studies have shown that there is no interference of sperm factor in results.

REFERENCES:


AmniSure ROM test package insert