LOURDES HOSPITAL
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LAB MANUAL

SUBJECT: **AmniSure** **ORIGIN DATE: 5/2009**
Rapid Fetal Membranes Rupture Test
REVIEWED: **7/31/2015** **REVISED: 3/2/12**

POLICY Rupture of membranes prior to 37 weeks' gestation complicates up to 12% of all pregnancies.The AmniSure ROM test is a rapid, non-instrumented, qualitative test for the detection of amniotic fluid in vaginal secretions of pregnant patients and will be used on those patients where Nitrazine and Fern Tests do not provide a definitive diagnosis of PROM.
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.

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 AmniSure 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 and can be read immediately or within 10 minutes. One line (Control) indicates no membranes are ruptured. Two lines indicate there is a rupture.

This method is classified as a "moderately complex" test procedure and is approved for use by Licensed Professionals, Nurses, Midwifes and Physicians. This test is used for definitive purposes.

Only authorized operators, those individuals who have attended a training session and successfully demonstrated the skills required for testing, may perform AmniSure testing. Operators will be competencied at 6 months after initial certification and yearly thereafter.

PROCEDURE:
<u>REAGENTS, MATERIALS, AND STORAGE REQUIREMENTS</u>

- AmniSure ROM Test Kit containing
  - AmniSure Test strip in foil pouch with desiccant
  - 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)
- AmniSure ROM Test Positive Control
- Saline
- Timer and Sample Rack

- Kit contents are for *in vitro* diagnostic use.

- Do not use kit contents after the expiration date printed on the outside of the kit.
- Do not use test strips if bent or damaged.
- Proper testing protocol must be followed to obtain accurate results

Storage Requirements:

- Store kits in dry location at room temperature 4-24 °C (40-75°F)
- Kits may be used until printed expiration date.
- Once AmniSure Test Strip is removed from foil pouch, it must be used **within 6 hours**.
- Amnisure ROM Test Positive Control stored in freezer -18°C to -12°C (0 to 10°F) for up to 18 months. Once reconstituted the solution is only stable for 24 hours stored in the refrigerator 2-10°C

SPECIMEN TYPE- A freshly collected sample of vaginal secretions

COLLECTION-

- Identify patient using full name and date of birth and position patient flat on back.
- 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**. (must leave swab in vagina for 1 minute)
- Place the swab in the properly labeled solvent vial and rotate for 1 minute. Remove and dispose of the swab in sharps container.
- 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 refrigerator for no more than 6 hours.

IDENTIFICATION and LABELING: the patient will be identified using 2 identifiers, patient full name and date of birth. All specimen containers and swabs will be labeled with patient name and date of birth

QUALITY CONTROL

External QC must be performed and validated by the Point of Care Coordinator or the Nurse Manager of Perinatal Services with each new shipment and or Lot# of AmniSure Test Kits or every 30 days if using the same lot# and shipment before any patient testing is performed.

Internal Controls

- Each AMNISURE test strip has built in reagent and procedural controls with an internal quality control line to ensure that adequate sample volume was present and adequate capillary migration of the sample has occurred.
- Any testing where the internal quality control line is not present cannot be interpreted. The AmniSure test strip may be defective or the sample volume may be inadequate Testing must be repeated with a new AMNISURE test strip.
- The appearance of the control line assures proper assembly of the strip that adequate sample volume was present and that adequate capillary migration (lateral flow) of the sample has occurred.

- A clear background in the result window is considered a negative internal control

External Controls:

- Prior to patient testing, external positive control containing PAMG-1 and negative control fluid containing no PAMG-1 are tested on each new lot # and/or shipment of AMNISURE test strips or every 30 days to ensure accurate test strip performance.
- 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 kept by the Laboratory Point of Care (x5802) in the Laboratory Hematology freezer.

Positive External Control-Procedure:

1. Take one AmniSure ROM Positive Control vial (contains 10 ng of freeze-dried Human PAMG-1 protein) from the freezer and add 1 ml of saline solution to it. Shake the resulting solution for a few seconds. Aliquot this solution into 5 separate vials (similar to the original vial) each containing 0.2 ml of the solution, so that 5 positive controls may be run from 1 vial of PAMG-1 positive control. The aliquots are stable for 24 hours when stored in the refrigerator at 4-8°C. Do not use after 24 hours.
2. Take one 0.2 ml aliquot of the positive control and dip the white end of the test strip into the vial for **exactly 10 minutes**.
3. Remove the test strip after exactly 10 minutes.
4. Read results by placing the test strip on a clean, dry, flat surface.
5. Do not interpret results **after 15 minutes have passed** since dipping test strip into vial.

Negative External Control - Procedure:

Place 0.2 ml of saline solution for the external negative control into a vial and follow steps 2 to 5 of the above procedure.

Control interpretation:

1 Line Present (control) in test area: Negative
2 Lines Present (control and Patient) in Test area: Positive
0 Lines Present in Test Area: Invalid Test

Note: If QC does not perform as expected:

- Repeat using a fresh QC sample
- Check operator technique, making sure proper timing was observed.
- Verify that test strips have been stored properly and are not bent.
- If expected results cannot be obtained, contact Point of Care Coordinator at x5802 or AmniSure Technical Support at 617-234-4441(www.amnisure.com)

TEST PERFORMANCE

1. Open the AmniSure test kit and remove the contents
2. Shake the solvent vial to make sure that all of the liquid in the vial settles to the bottom
3. Open the solvent vial and place it in a vertical position
4. Insert the sterile polyester swab from the AmniSure test kit into the vagina 2-3 inches.
5. Withdraw the swab after one minute has elapsed
6. Place the polyester swab into the vial
7. Rinse the swab by rotating for one minute
8. Remove and dispose of the swab after one minute
9. Tear open the foil pouch at the test notches and remove the AmniSure test strip.
10. Dip the white end of the test strip (marked with arrows) into the solvent Allow strip

to remain in vial for 10 minutes. A positive result may be read after 5 minutes.

NOTE: Strong leakage of amniotic fluid will make results visible within minutes, while a small leak may take up to 10 minutes.

11. Read the results by placing the strip on a clean, dry flat surface.

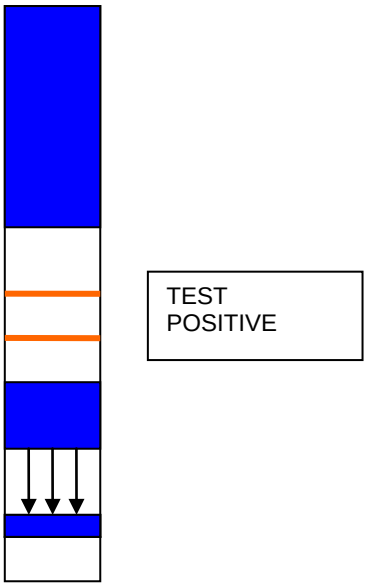
Do <u>not</u> read or interpret results <u>after 15 minutes have passed</u> since placing test strip into vial.

12. Record patient results on the log sheet.

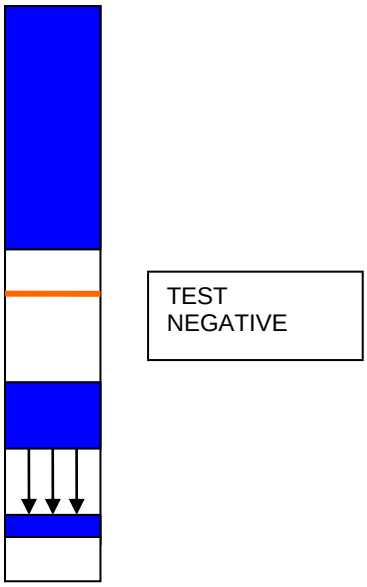
RESULT INTERPRETATION

There are 3 possible result interpretations:

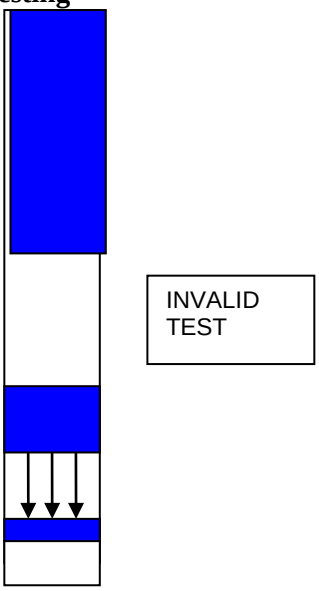
Control Line and Test Line Present In Test Area: Positive for Rupture of Membrane



Only Control Line Present in Test Area: Negative for Rupture of Membrane



Control Line Not Present in Test Area: INVALID TEST 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.

Expected Values and Clinical Significance:

The vaginal secretions of a patient with the fetal membranes intact contains no PAMG-1 and will test negative with the AmniSure Test. Clinically significant leakage of amniotic fluid increases PAMG-1 concentration in cervico-vaginal secretions by a factor of thousands resulting in a positive AmniSure Test.

Result Reporting

Patient results are not reported unless the quality control is acceptable.
Results are reported as Positive for Rupture of Membrane or Negative for Rupture of Membrane

The patient's results must be recorded in the patients chart as well as the accession log with patient's name, date of birth, the date and time of the test, and signature of the person performing the test. The accession record must also have the AmniSure kit lot number and expiration date.

Interfering Substances

- 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.
- 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.

Procedural Limitations

- 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.
- 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.
- 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.
- Test strips must remain sealed in foil pouch until just prior to use. Once open, the test strip must be used within 6 hours
- Do Not interpret results after 15 minutes have passed since placing test strip in vial.
- Positive test results from a strong leakage of amniotic fluid may be visible right away. A very small leak of amniotic fluid may take the full 10 minutes to become positive. A negative result cannot be interpreted until the full 10 minutes have elapsed.

References:

[1] Excerpt from "Technical Innovations in Clinical Obstetrics," Joong Shin Park and Errol Norwitz. Contemporary OB/GYN, September 15, 2005, vol. 50.

Cousins LM et al., "AmniSure Placental Alpha Microglobulin-1 Rapid Immunoassay versus Standard Diagnostic Methods for Detection of Rupture of Membranes", American Journal of Perinatology, Volume 22, 2005.

AmniSure ROM test package insert 02/05/09.

Next Review:

AUTHORIZATIONS:

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Medical Director Date

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Point of Care Coordinator Date

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Nurse Manager, Perinatal Services Date