HealthPartners/GHI

Amnisure® Rupture of Fetal Membranes (ROM) Test Procedure	**Attachments** ☒ Yes ☐ No
Key words AmniSure®, Rupture of Fetal Membranes, Amniotic Fluid, PAMG-1 (placental a-1 microglobulin) protein	**Number** GHP-PC-CLINIC LAB-Procedures-Amnisure® v. 06-2013
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	Retired Date
Level of Complexity: Non-waived	
Review Responsibility Laboratory Technical consultants	**Contact** Laboratory Technical Consultants
APPROVAL(S) Laboratory Medical Director	

Amnisure procedure
Clinic Lab Procedure (Pages 1-5)
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I. PURPOSE/PRINCIPLE

The AmniSure® 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.

AmniSure® is approved for use at any gestational age. 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.

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 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.

Reagents and Equipment:

- AmniSure AmniSure® 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)
- Saline or Distilled Water
- Timer and Sample Rack

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 AmniSure Test Strip is removed from foil pouch, it must be used **within 6 hours**.

Specimen:

Nursing Procedure:

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.
- Notify the lab of the need for an Amnisure test. The lab will bring the Amnisure swab from the Amnisure kit to the care unit. Use the swab from the Amnisure Kit. Substitutes are not acceptable.
- Position patient flat on back.
- 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.**
- Label with a patient label.
- Hand the swab to the laboratory technician.

Quality Control:

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:

- QC will be performed monthly and with changes to lot numbers.

- 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.

Equivalent Quality Control:
The AmniSure® procedure is a testing system that qualifies for Equivalent Quality Control since there is an internal quality control mechanism which monitors all sources of error..

This procedure consists of performing both internal QC and two levels of external QC for 10 consecutive testing days. If all results are acceptable for both internal and external QC the laboratory may then perform and document internal QC daily and perform and document external QC once per month and with each new lot number.

If any of the following occurs, the lab must stop using the equivalent QC protocol until corrective actions have been completed and documented.

- Either internal or external QC failures that are not resolved by repeating the control one time
- PT failure
- Any problems identified with this testing as a result of analytic Quality assessment activities.

The laboratory must revert to performing two levels of external QC every day of patient testing while troubleshooting and solving the problem.
Another Equivalent Quality Control study must be performed before resuming Equivalent Quality Control Procedures.

Quality Control Procedure:
- External Positive Control: Human amniotic fluid, diluted 1:100 with Saline or Distilled Water
- External Negative Control: Saline solution.
- Human amniotic fluid can be stored frozen in a dry place at –18 to -12°C for up to 18 months.
- 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.
- Pre-diluted controls may be frozen until needed.

External Positive Control – Procedure:
1. Controls are kept in the freezer. Remove one and allow it to thaw.
2. After control is thawed, mix thoroughly.
3. Dip the white end of the test strip into the control vial **for exactly 10 minutes**.
4. Remove the test strip after exactly 10 minutes.
5. Read results by placing the test strip on a clean, dry, flat surface.
6. Do not interpret results **after 15 minutes have passed** since dipping test strip into vial.

External Negative Control – Procedure:
Using saline solution (or distilled water) for the external negative control, follow steps 3 to 6 of the above procedure. Document external QC on the AmniSure® worksheet.

If External Quality Control Does Not Perform As Expected:
- Repeat testing on newly diluted positive QC or fresh tube of saline solution.
- Check operator technique, making sure proper timing is observed.
- Make sure test strips have not been stored improperly and are not bent.
- If expected results are not obtained, contact AmniSure® Technical Service at 617-234-4441 (www.amnisure.com) See POCT/Lab Resource, click on AmniSure® Procedural Checklist 2008

Expected Results:
1 Line Present (Control) in Test Area: Negative for Ruptured Membranes
2 Lines Present (Control and Patient) in Test Area: Positive for Ruptured Membranes

0 Lines Present in Test Area: Invalid Test

III. PROCEDURE

On the Care Unit:

1. Care unit staff: Notify the laboratory of the Amnisure order.
2. Lab tech: Take the AmniSure® kit and a timer to the Care unit. Open the AmniSure® kit. Remove the swab and give to the Care Unit staff. Label the blue-capped solvent vial from the kit with patient information.
3. Care Unit staff: After specimen collection, give the swab to the lab tech for testing.
4. Lab tech: Hold the solvent vial by the cap and shake well to ensure all liquid in vial is on bottom of vial.
5. Open vial and 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.
6. Remove and dispose of the swab according to standard precautions.

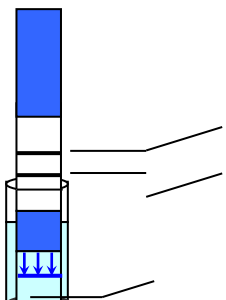
In the lab:

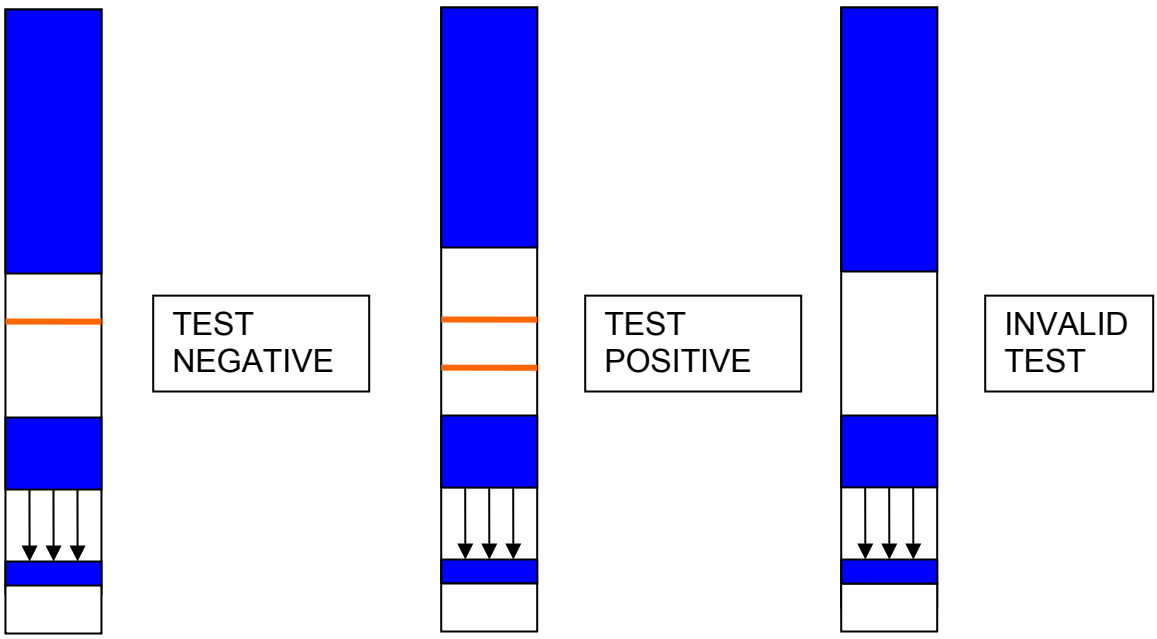
7. Tear open foil pouch at the tear notches and remove AmniSure® test strip.
8. Dip white end of strip (marked with arrows) into the correct, labeled vial of solvent.
9. Allow strip to remain in vial for 5-10 minutes, unless 2 lines are clearly visible.

NOTE: Strong leakage of amniotic fluid will make results visible after 5minutes, while a small leak may take up to 10 minutes. Results can be reported as soon as two lines appear in the test region, which sometimes occurs within seconds.

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

NOTE: Requires a full ten minutes in order to ensure accurate reporting interpretation.
Do <u>not</u> read or interpret results <u>after 15 minutes have passed</u> since placing test strip into vial.





CALCULATIONS
NA

EXPECTED VALUES/REFERENCE RANGE
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 THE PROCEDURE
- 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.
- AmniSure® is approved for use at any gestational age.
- 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.
- 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 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.
- False negative results and delay in the diagnosis of rupture of membranes can increase the risk of chorioamnionitis, oligohydramnios and umbilical cord accident.
- 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.

SPECIAL SAFETY PRECAUTIONS
Use Universal Blood and Body fluid Precautions.

PROCEDURE NOTES
- To obtain a minimal PAMG-1 concentration, dilute human amniotic fluid 1:100 with either saline or distilled water. Since this is a qualitative test, this can be done by using 99 drops of saline or distilled water and1 drop of amniotic fluid. Mix for a few seconds. Transfer approximately 20 drops of the dilution to a vial similar to the vial supplied in the AmniSure® test kit. Each vial is labeled with an expiration date.
- Controls are available through Central lab

REFERENCES
AmniSure® ROM test package insert, August 2007; AmniSure Intl. LLC.

RELATED DOCUMENTS
NA

APPENDIXES
NA

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IV. DEFINITIONS

V. COMPLIANCE
Failure to comply with this policy or the procedures may result in disciplinary action, up to and including termination.

VI. ATTACHMENTS

(www.amnisure.com)

VII. OTHER RESOURCES

VIII. ENDORSEMENT

Laboratory Administration

Computer Order and Result Entry

Rupture of Fetal Membranes Amnisure ROM

Order Code: RUPFM

ORDERING

Order code RUPFM

RESULTING

WORKSHEET:

Function MEM Worksheet CM_ _ (Clinic Micro)

CODE	NAME	RESPONSE
ROM	Fetal ROM	POS or NEG
IQC	Internal QC	Automatically resulted with HIDE
ISID2	Operator ID 2	Automatically resulted with HIDE

RECORDING RESULTS FOR RUPFM

1) Use Daily Worksheet to record results.
2) Immediately enter ROM results into computer, so results are electronically available.

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