Ruptured Fetal Membrane - AmniSure (LTR39872)

Folder Name: POCT\04 POCT-Non-waived Procedures

Approval Workgroup: POCT

Revision: 1

Next Review Date:

Last Approved By: Benirschke, Robert (11/4/2015 2:43:15 PM)

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BACKGROUND INFORMATION

These are the instructions for the CLIA moderately complex test for AmniSURE.

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 secretions of pregnant women. AmniSure detects PAMG-1 (placental microglobulin) protein marker of the amniotic fluid in vaginal secretions.

MANUFACTURER

This method is manufactured by AmniSURE International, LLC. It is performed as per the manufacturer's instructions, without any in-house modifications.

OSHA SAFETY CLASSIFICATION

This procedure is classified as a Category I potential hazard by OSHA standards. Appropriate barrier equipment and precautions must be used in performing the procedure.

CLIA COMPLEXITY

Moderate

SUMMARY AND EXPLANATION OF TEST

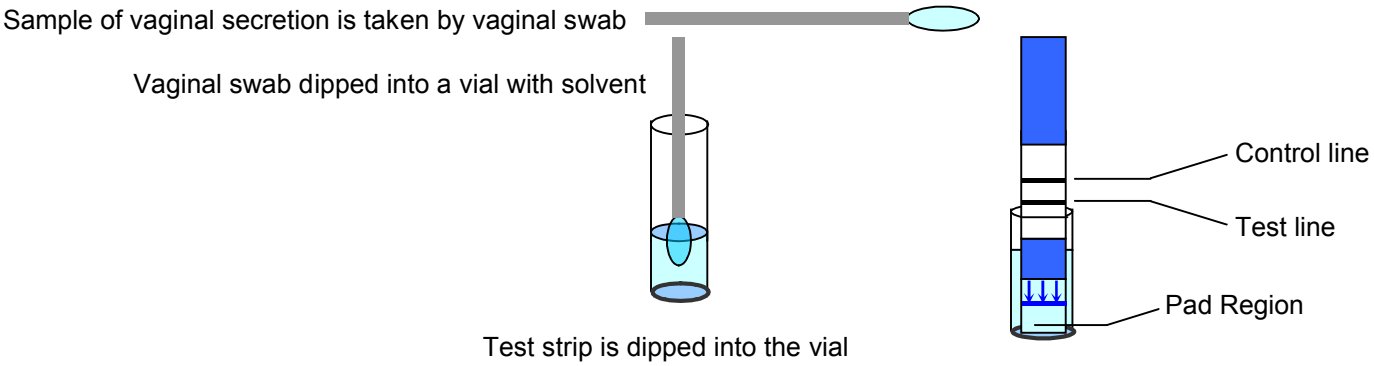
The timely and accurate diagnosis of rupture of [fetal] membranes (ROM) is crucial since the ROM may be associated with serious neonatal and maternal consequences. Failure to identify patients with ROM can result in the failure to intervene appropriately. Conversely, the false diagnosis of ROM can lead 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. Accurate diagnosis of [fetal] membranes rupture, however, remains a frequent clinical problem in obstetrics.

In a clinical trial, the AmniSure test correlated with clinical diagnosis obtained through combined usage of three routinely used tests (Nitrazine, Ferning, and Pooling).

PRINCIPLE OF TEST

The test does not require the traditional speculum examination routinely used for ROM diagnosis. A sample of amniotic fluid (acquired via vaginal swab) is diluted into a vial containing solvent. The solvent

extracts the sample from the swab by rinsing 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 over the next 5-10 minutes by the presence of one or two lines. One line indicates no membranes ruptured, two lines indicates there is a rupture.



The AmniSure® ROM Test uses the principles of immunochromatography to detect human PAMG-1 (placental α microglobulin-1) 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 secretions 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 secretions 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 extremely small quantities of amniotic fluid in vaginal secretions. Background concentration of PAMG-1 is around 50-220 picogram (i.e. 0.05-0.22 ng) per 1ml of vaginal secretion. The sensitivity cut-off of AmniSure® is 5 ng/ml, i.e. at least 20 times higher than the background concentration.

During the test procedure, placental microglobulin from the sample sequentially binds to a 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” the PAMG-1 bound conjugated antibodies flowing up from the Pad Region. This “meeting” immobilizes the system of PAMG-1 bound 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 correctly. This line appears when rabbit anti-mouse IgG antibody ‘catches’ the mouse antibody with gold dye. Gold dye gives the resulting line its color.

SPECIMEN REQUIREMENTS

Specimen Collection and Handling:

- Collect sample of vaginal secretions using sterile vaginal swab, rinse it in the solvent vial, and dispose of the swab as indicated in the test procedure section (see below).
- Run the patient sample as soon as possible.
- Samples must be run within 30 minutes of collection. If a sample can not be run within 30 minutes and sample storage is necessary, tightly close the sample vial and place it in refrigerator for up to 6 hours.

MATERIALS AND EQUIPMENT NEEDED

1. AmniSure Test Strip (lateral flow device) in foil pouch with desiccant.
   Each Test strip contains:
   - Monoclonal antibody (produced by mouse hybridoma)
   - Immobilized monoclonal antibody (produced by a different mouse hybridoma)
   - Colloidal gold particles linked to monoclonal antibody
   - Mouse IgG labeled with colloidal gold particles
   - Rabbit (or goat) anti-mouse IgG antibody
2. Sterile polyester vaginal swab
3. Plastic vial with water solvent. Solvent solution contains:
   - 0.9% NaCl
   - 0.01% Triton X100
   - 0.01% NaN_3 (Azide Na)
4. Timer

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

- Store the kit in a dry place at room temperature or under refrigeration at 4 to 24^{o}C (40 to 75^{o}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.

REACTIVE INGREDIENTS

1. Urea, USP, 29 mg/mL per sample well.
2. Phenol red (pH indicator)
3. Bacteriostatic agents

CALIBRATION

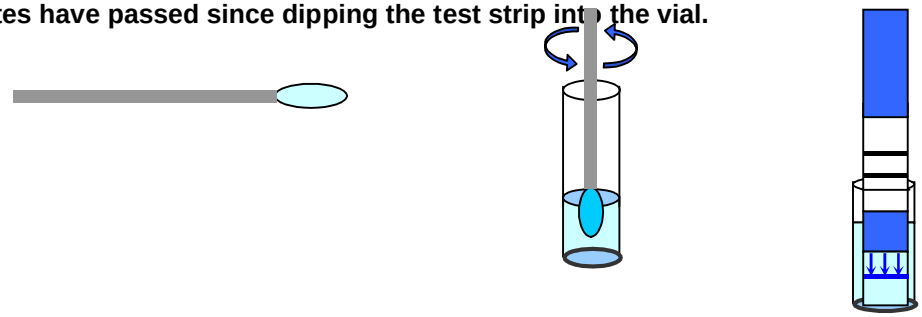
Not required

PROCEDURE

1. Take the solvent vial by its cap and shake well to make sure all liquid in the vial settles to the bottom. Open the solvent vial and stand it upright.
2. To collect a sample from the surface of the vagina, use the sterile Polyester swab provided. Remove the sterile swab from its packaging following instructions on the pack. The Polyester tip should not touch anything prior to its insertion into the vagina. Hold the swab in the middle of the stick and, while patient is lying flat on her back, carefully insert the Polyester tip of the swab into the vagina 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.

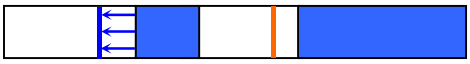
6. Dip the white end of the test strip (marked with arrows) into the vial with solvent. Strong leakage of amniotic fluid may make the results visible early (within 5 minutes), while a very small leak will take the full 10 minutes.

7. Remove the test strip if two lines 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.**



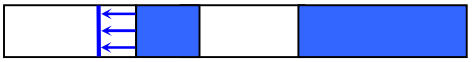
INTERPRETATION OF RESULTS
There are three possible results: (a) Positive Result (b) Negative Result (c) Invalid Result

One line, NO MEMBRANES RUPTURED



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.

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 line coloration.

- **Positive Result:** The generation of two (2) lines indicates a positive result. A positive result is indicative of the presence of amniotic fluid in vaginal secretions. There is a rupture.
- **Negative Result:** The generation of one (1) line indicates a negative result. A negative result is indicative of the absence of amniotic fluid. No membranes rupture.
- **Invalid Result:** If at 10 minutes no lines appear, the test is considered invalid. If the test result is invalid, repeat the test with a new patient sample and new AmniSure test.

CALCULATIONS

No user performed calculations are needed for this method.

QUALITY CONTROL

Internal Quality Control
Each AmniSure test has a 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. The appearance of the *control line* assures adequate sample volume, adequate capillary migration of the sample has occurred, and the presence of antibodies on the test strip. It also verifies proper assembly of the test strip. This covers the analytic components of the test.

External Quality Control Testing
The freeze dried external controls will be stored at a temperature between 2 to 25 C in the Department of Pathology and Laboratory Medicine. External controls must run to insure the integrity of the assay and the control testing frequency is as follows:

1. External controls must be run on each new lot of reagents/test strips before use on patient samples.
2. External controls must be run with each new shipment of reagents/strips before use on patient samples.
3. External controls must be run at a minimum of every 30 days.

Positive Controls:
Freeze-dried PAMG-1 protein, concentration of 10.0 ng/ml. The minimum PAMG-1 concentration in amniotic fluid is 2,000 ng/ml.

Negative Controls:
Saline solution (AmniSure® Negative Control (AI0013) is recommended for the negative external control. Follow last two steps of the procedure for the positive control to perform negative control testing.

Control Preparation and Testing

1. Take the vial containing 10 ng of freeze-dried Human PAMG-1 protein (positive control) and pour the contents of the solvent solution (1mL) into the positive control vial. Shake the resulting solution for few seconds.
2. Once prepared the positive control is stable for 24 hours if stored in the refrigerator at 2-8 C.
3. Prepare the negative control in the same manner as the positive control. Pour the contents of the solvent solution into the negative control. Seal the vial and shake it for a few seconds.
4. Use the solution from step #1 for positive quality control of the AmniSure® ROM (Rupture Of fetal Membranes) and the Negative control from step #3 follow the steps below:
    a. Aliquot 200 ul of the control solutions into separate plastic test tubes, labeling one positive and the other negative.
    b. Dip the white end of the test strip (marked with arrows) into the positive control solution from step #2 **for 10 minutes sharp**.
    c. Remove the test strip 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.**

See the test procedure above for interpretation of the quality control testing results.

Should be external quality control fail, repeat the quality control testing to insure there was not a procedural problem. If the quality control fails the second time, sequester the reagents/test strip and notify the nurse manager. **DO NOT USE** the testing strips on any patient samples. The nurse manager will work with the Point of Care personnel to resolve any problems with the manufacturer.

STORAGE AND STABILITY OF CONTROL MATERIAL

Freeze-dried purified PAMG-1 can be stored in a dry place at 2 to 25 C until the expiration date.

After dissolving the freeze-dried PAMG-1 with the saline solution, the obtained solution can be stored

under refrigeration at +4 to +8°C (39 to 46°F) for up to 24 hours.

RESULTS REPORTING

Follow the nursing reporting guidelines. Test Code AMNIS Lab Order LAB2453 CPT: 84112

Document in Doc Flowsheets "Labor Singleton" >Cervical Exam>Amnisure ROM put result at time performed> then put in order for Lab2453 AMNIS.

REFERENCE RANGE

N/A

PROCEDURE NOTES

AmniSure is classified by the FDA as a CLIA waived test and it has been approved for use by physicians, nurses, and nurse midwives

LIMITATIONS

1. Specimen collection is a critical factor for accuracy of testing results.
2. The assay must be run within 30 minutes of vaginal specimen collection.
3. Trace amounts of blood do not interfere with test results.
4. Insure sample collection is not compromised by vaginal detergents, digital exam, KY jelly, or other substances/procedures that may impede sample collection.

ALTERNATIVE METHOD

Not applicable

REFERENCES

AmniSURE ROM Test procedure manual, September 2009.

For technical assistance, AmniSure® Intl. LLC may be contacted at Tel. 617-234-4441 or email to info@amnisure.com

EFFECTIVE DATE

The effective date for this procedure is November 23, 2009.

WRITTEN BY

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