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Area	Laboratory-Point of Care
Applicability	Dearborn, Troy

AmniSure Rupture of Fetal Membranes (ROM)

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

A. To describe how to perform the AmniSure Rupture of Fetal Membranes (ROM) testing at the point of care (POC) and to provide troubleshooting instructions to non-laboratory testing personnel.

B. This document is only applicable to areas that are approved for testing under one of the laboratory's Clinical Laboratory Improvement Amendments (CLIA) certificates.

II. PRINCIPLE AND CLINICAL SIGNIFICANCE:

A. The AmniSure ROM test is a rapid, non-instrumented, qualitative immunochromatographic test for the *in vitro* detection of amniotic fluid in vaginal discharge of pregnant patients. 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 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, two, or no lines. The presence of only a control line indicates no membranes ruptured. Two lines indicate a rupture. A test line only or no lines indicate an invalid test.

B. The AmniSure ROM test detects PAMG-1 (placental alpha microglobulin-1) protein marker of the amniotic fluid in vaginal discharge of patients who report signs, symptoms, or complaints suggestive of ROM. Placental microglobulin was selected as a marker of fetal membranes rupture due to its unique characteristics (i.e. its high level in amniotic fluid, low level in blood, and extremely low background level in cervico-vaginal discharge when the fetal membranes are intact). To minimize the frequency of false results, two monoclonal antibodies have been selected to set the sensitivity threshold of the AmniSure test at the optimal low level. This level allows the detection of extremely small quantities when anti-mouse IgG antibody "catches" the mouse antibody with gold dye. The gold dye gives the resulting line its color.

C. The timely and accurate diagnosis of ROM is crucial because of ROM may be associated with serious neonatal and maternal consequences. Failure to identify patients with ROM can result in

the failure to intervene appropriately.

D. 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.

III. SPECIMEN COLLECTION AND HANDLING:

Always follow established procedures for Standard Precautions/Hand Hygiene when collecting and handling a specimen. Hands must be washed or disinfected with antiseptic soap or an alcohol-based hand rub as outlined in the Laboratory Infection Control policy before and after gloves are used. Gloves must be worn when performing patient testing and changed between patients.

A. Patient Preparation
    1. Patients should avoid disinfectant solutions or medicines in the vagina 6 hours prior to specimen collection.

B. Patient Identification
    1. Patients must be identified at the bedside using two identifiers (Joint Commission).

C. Specimen Labeling
    1. Testing should be performed at the patient's bedside, when possible. If the specimen is taken to another location for patient testing, the specimen must be labeled with the patient name and identification (ID) number (Joint Commission).

D. Specimen Types
    1. Vaginal specimen collected with sterile polyester swab provided in the AmniSure kit.

E. Specimen Collection, Handling, Transportation, and Processing
    1. Verify that the specimen collection swab is not expired.
    2. Remove the swab from the package. The swab should not touch anything prior to insertion into the vagina.
    3. Hold the swab in the middle of its shaft and, with the patient lying supine, carefully insert the polyester tip of the swab into the vagina until the fingers contact the skin (no more than 2-3 inches).
    4. Leave the swab in the vagina for 1 full minute.
    5. Test the sample immediately after specimen collection.
        a. If the swab must be taken away from the bedside for testing, place the swab in a clean conical tube with the patient's name and ID on the tube.

F. Specimen Storage
    1. If testing cannot be performed immediately, the inoculated, labeled solvent vial may be sealed and stored for 4 hours after collection at room temperature. The solvent vial may be stored refrigerated up to 6 hours after collection. See the Procedure section below for steps of how to prepare the solvent vial.

G. Specimen Acceptability Criteria
    1. Vaginal specimen collected using the sterile polyester swab provided with the kit

H. Specimen Rejection Criteria

1. Specimens collected from a patient that used disinfectant solutions or medicines in the vagina less than 6 hours prior to specimen collection
2. Swabs that are contaminated by foreign objects/body parts prior to insertion or removal from the vagina
3. Specimens collected using a swab other than what is provided in the kit
4. Unlabeled specimens taken from the bedside to another location for testing

IV. REAGENTS:

A. AmniSure ROM Test Kit (FMRT-1-25 for 25 test kit, FMRT-1-10 for 10 test kit)

1. Contents
    a. Instructions for use
    b. Test strips stored in a foil pouch with desiccant
    c. Sterile polyester specimen collection swabs
    d. Vials containing test solvent
2. Availability
    a. Dearborn: Ordered and stored in testing area.
    b. Troy: Kits are ordered by Decentralized Testing. POC staff distributes kits to the testing area for use.
3. Ingredients
    a. The solvent contains 0.9% sodium chloride, 0.01% Triton X-100, 0.05% NaN_3
4. Storage and Handling
    a. Store kits in dry location at room temperature 4-25°C (40-77°F).
    b. Do not freeze.
5. Expiration
    a. When stored in the foil pouch at the recommended temperature, the test is stable until the "Use By" date printed on the foil pouch.
    b. Use the test within six hours after removing from the foil pouch.
6. Warnings/Precautions
    a. For *in vitro* diagnostic use only.
    b. Do not interchange components from other kit lots.

B. AmniSure ROM Test Positive Control

1. Contents
    a. 1 Positive Control Vial
    b. 1 AmniSure Solvent Vial
2. Availability

a. Dearborn: Ordered and stored in testing area.

b. Troy: QC is ordered by Decentralized Testing. POC staff distributes QC to the testing area for use.

3. Ingredients

a. Positive Control: 10 ng of PAMG-1 protein purified from human amniotic fluid and lyophilized with buffered saline.

b. Solvent: Water-based solution containing distilled water, 0.9% sodium chloride, 0.01% Triton X100, 0.05 sodium azide.

4. Storage and Handling

a. Store in a dry place at 2-25°C (35-77°F).

b. Do not freeze.

c. Reconstituted controls can be refrigerated for up to 24 hours at 4-8°C (39-46°F).

5. Expiration

a. Do not use beyond the expiration date printed on the vial.

6. Warnings/ Precautions

a. For *in vitro* diagnostic use only.

b. Each control is a single use disposable unit. Components cannot be reused.

C. AmniSure ROM Test Negative Control

1. Contents

a. 1 Negative Control Vial

b. 1 AmniSure Solvent Vial

2. Availability

a. Dearborn: Ordered and stored in testing area.

b. Troy: QC is ordered by Decentralized Testing. POC staff distributes QC to the testing area for use.

3. Ingredients

a. Negative Control: Sucrose solution.

b. Solvent: Water-based solution containing distilled water, 0.9% sodium chloride, 0.01% Triton X100, 0.05 sodium azide.

4. Storage and Handling

a. Store in a dry place at 2-25°C (35-77°F).

5. Expiration

a. Do not use beyond the expiration date printed on the vial.

6. Warnings/ Precautions

a. For *in vitro* diagnostic use only.

b. Each control is a single use disposable unit. Components cannot be reused.

V. EQUIPMENT AND SUPPLIES:

A. Timer or Clock

B. Sample Rack

VI. QUALITY CONTROL (QC):

A. Each AmniSure test has built-in (internal) reagent and procedural controls to confirm accurate reading of the results. The appearance of one line in the control region of the test result area verifies the integrity of the test procedure.

B. Additionally, two levels external controls are performed.

1. External QC Testing Frequency
    a. Both levels are performed with each new lot of kit.
    b. Kits are also tested monthly when strips are stored for more than 30 days.
    c. QC will be performed to confirm test performance when test results do not correlate with the patient's clinical presentation.

2. QC Procedure
    a. Internal QC is performed with each test to verify adequate sample volume, confirm capillary migration occurred, and proper procedural steps were followed.
        i. Testing personnel will view each strip to verify that the control line is present. If the control line is not present, the test is invalid and should be repeated.
    b. External QC Procedure
        i. Uncap the AmniSure solvent (liquid) vial.
        ii. Uncap the positive control (freeze-dried) vial.
        iii. Add the solvent (liquid) to the positive control (freeze-dried) vial.
            a. Note: The polyester swab is not needed for the external QC procedure.
        iv. Cap the solution and mix by shaking vigorously for a full 30 seconds to allow for full reconstitution.
        v. Obtain an AmniSure test strip and open the foil pouch to remove the strip.
        vi. Insert the white end of the test strip into the control solution.
            a. Note: The arrows on the test strip should be pointing down when introduced in the solution.
        vii. Allow the test strip to remain in the solution for 5 minutes.
            a. Do not read or interpret the results after 10 minutes have passed since first dipping the test strip into the vial.

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

ix. Document the external QC on the POC AmniSure Monthly QC / New Lot QC Validation form.

x. Repeat the External QC Procedure steps for the negative control.

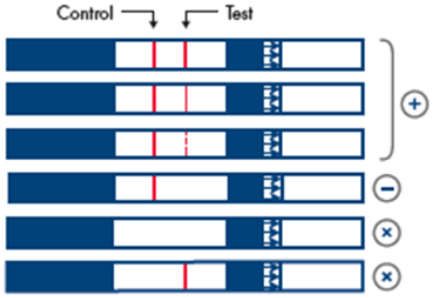
xi. Discard the test strips and vials in a biohazard or biohazard sharps container.

3. Expected QC Results

   a. The control line must be present for each patient and QC run. If the control line is not present, the test is invalid (x).

   b. The control line and test line must be present for the positive external control (+).

   c. Only the control line must be present for the negative external control (-).



AmniSure® ROM Test Quick Reference Guide

4. QC Failure Procedure

   a. If one or more parameters is out of the acceptable range, appropriate corrective action must be taken and documented. Patient testing must be discontinued until the problem is resolved.

   b. If unacceptable QC results appear, the following items should be verified:

      i. Verify that the QC and strips are not past their expiration dates.

      ii. Confirm proper temperature storage for QC and strips.

      iii. Observe the strip for defects (such as bending).

      iv. Verify that the control/solvent solution was mixed well prior to testing.

      v. Confirm that the proper procedure and technique were followed.

      vi. Repeat the test using new QC and/or new strips.

   c. If none of the items above resolves the issue, contact the site-specific POC department.

      i. Dearborn: Call POC at 313-436-2367, 313-593-7970 or 313-982-5661.

      ii. Troy: Call Decentralized Testing at 248-964-8009.

VII. PROCEDURE:

A. Open AmniSure kit, remove and label the solvent vial with the patient name and ID.

B. Hold the solvent vial by the cap and shake well. Verify that all of the liquid in the vial is at the bottom.

C. Open solvent vial and place into a rack to keep vertical.

D. Collect the patient sample as indicated in the Specimen Collection and Handling section above.

E. After removing the swab from the vagina, immediately place the polyester tip into the labeled solvent vial and rotate the swab for 1 minute.

F. Remove and dispose of the swab in a biohazard container or biohazard sharps container.

1. Test the sample within 4 hours of collection. If the sample is not tested within 4 hours, tightly close the labeled sample vial and place in a refrigerator at 2-8 °C. Do not test the sample after more than 6 hours have passed since sample collection.

G. Tear open foil pouch at the tear notches and remove AmniSure test strip.

1. Use the AmniSure test within 6 hours after removing from the foil pouch.

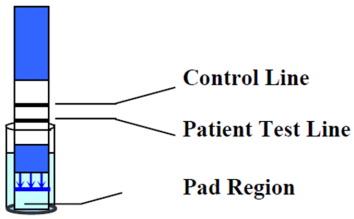
H. Dip white end of the test strip (marked with arrows facing downward) into the vial with the solvent.

I. Allow strip to remain in vial for 10 minutes.

1. Strong leakage of amniotic fluid will make the results visible before the 10 minute incubation is complete, while a small leak may take up to 10 minutes.

J. Remove the test strip from the vial.

K. Read the results by placing the strip on a clean, dry, flat surface in a well-lit environment via either natural light or fluorescent light.



AmniSure® ROM Test Instructions for Use

1. Do not read or interpret results after 15 minutes have passed since placing test strip into vial.

L. To help with correct identification of test and control line locations, compare the test strip to the graphics on the outer kit bag or foil pouch.

M. Discard the vial, swab, and test strip in a biohazard container or biohazard sharps container.

VIII. INTERPRETATION OF RESULTS:

A. Positive

1. A positive result is indicated by two lines in the test region.

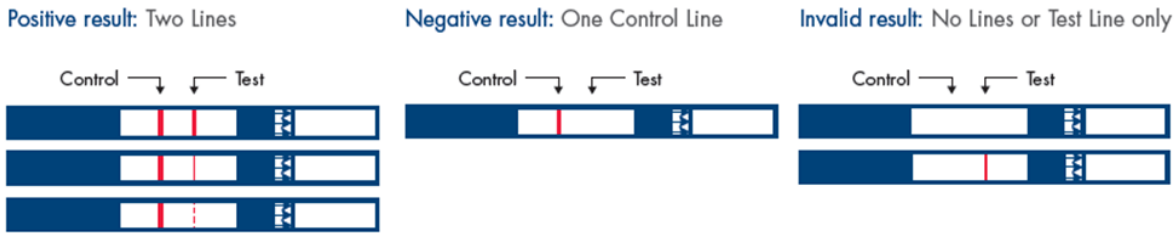
B. Negative

1. A negative result is indicated by the presence of a control line and no test line.

C. Invalid

1. The presence of no lines or only a test line indicates an invalid test result. Do no interpret this as a negative result. Invalid tests must not be reported or acted upon clinically.

D.



AmniSure® ROM Test Quick Reference Guide

1. The intensity of the lines may vary. The test is valid even if the lines are faint or uneven. Do not interpret the test result based on the intensity of the lines.
2. Negative AmniSure results alone may not rule-out membrane rupture. This test may be used in conjunction with other ROM tests such as pHizatest and Fern Test, where available.

IX. EXPECTED VALUES:

Leakage of amniotic fluid is indicative of the (fetal) membranes rupture in patients.

X. RESULT REPORTING:

All AmniSure patient results must be documented in the patient's chart in the hospital information system (HIS) and must be accompanied by the internal QC result, date and time of specimen collection and testing, and testing personnel identification.

XI. REPORTABLE VALUES:

A. Negative: No Membrane Rupture.

B. Positive: Rupture.

XII. SYSTEM DOWNTIME:

If the HIS is down, follow the department's downtime procedure and verify that all notes are documented on paper chart for later result entering/scanning when the system becomes available. Verify that date/time of test, result, and QC outcome, and testing personnel are documented.

XIII. LIMITATIONS:

A. A false negative test may result in an inadequate level of care for newborns less than 37 weeks gestation.

B. False negative results can delay the diagnosis of rupture of membranes and can increase the risk of chorioamnioitis, oligohydramnios, and fetal umbilical cord accident.

C. Interrupted leakage with minimal residual fluid can lead to a false negative result.

D. Until the diagnosis of membrane rupture is excluded, avoid digital cervical examination to prevent infection and shorten the latency period. Placenta previa and performing digital exams prior to specimen collection can lead to inaccurate test results.

E. Do not use damaged components of the test kit.

F. Do not reuse the test kit components.

G. Do not bend or fold the test strip or the aluminum foil pouch with the test strip in it.

H. The AmniSure test results are qualitative. Make no quantitative interpretation based on the test results.

I. When there is a significant presence of blood on the swab, the test can malfunction and testing is not recommended. In cases of trace amounts of blood on the swab, the test still functions properly.

J. In 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.

K. Test performance in patients without signs or symptoms of ROM is unknown.

L. Use results in conjunction with other clinical information.

M. Failure to detect membrane rupture does not assure the absence of membrane rupture.

N. Patients may labor spontaneously despite a negative test result.

O. Components from other AmniSure kits and/or lot numbers are not interchangeable.

XIV. INTERFERING SUBSTANCES:

A. Vaginal infections or urine do not interfere with the results of the AmniSure test.

B. Studies have shown that there is no interference of sperm factor in results.

C. The performance of the AmniSure test has not been established in the presence of the following contaminants: anti-fungal creams or suppositories, K-Y® Jelly, Monistat® Yeast Infection Treatment, baby powder (starch and talc), Replens® Feminine Moisturizer, or baby oil.

D. The performance of the AmniSure test has not been established in the presence of meconium in the amniotic fluid.

XV. TROUBLESHOOTING:

A. AmniSure results are affected by poor technique during specimen collection and test procedure. The accuracy of the test is largely dependent upon the quality of the sample collection and processing. Results may be affected by any of the following:

1. Poor collection technique
2. Contamination or dilution of the specimen

B. Any result exhibiting inconsistency with the patient's clinical status should be repeated or supplemented with additional testing, at the clinician's discretion.

XVI. REFERENCES:

A. Point of Care Testing Approval Process

B. The Joint Commission. (2022) Standard NPSG.01.01.01 EP 1 in The Joint Commission. Comprehensive accreditation manual. Hospital edition. Oak Brook, IL: The Joint Commission.
C. AmniSure® ROM Test Instructions for Use, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1090607 Rev. 06 02/2022. www.qiagen.com/us.
D. AmniSure® ROM Test Quick Reference Guide, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1121020 02/2020. www.qiagen.com/us.
E. AmniSure® ROM Test Positive Control, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1091028 Rev. 02 3/21/2017. www.qiagen.com/us.
F. AmniSure® ROM Test Negative Control, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1091028 Rev. 02 3/21/2017. www.qiagen.com/us.

Attachments

AmniSure Monthly QC.pdf

AmniSure New Lot Number Validation.pdf

AmniSure Patient and Internal QC Log.pdf

AmniSure Training and Competency Assessment.pdf

AmniSure Training Guide.pdf

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Approval Signatures

Step Description	Approver	Date
Medical Director	Vaishali Pansare: Chief, Pathology	7/7/2023
Medical Director	Jeremy Powers: Chief, Pathology	6/29/2023
Policy and Forms Steering Committee Approval (if needed)	Jessica Czinder: Mgr, Division Laboratory	6/12/2023
POC Best Practices	Nga Yeung Tang: Tech Dir, Clin Chemistry, Path	6/9/2023
POC Best Practices	Caitlin Schein: Staff Physician	5/25/2023
POC Best Practices	Ann Marie Blenc: System Med Dir, Hematopath	5/24/2023

	Jessica Czinder: Mgr, Division Laboratory	5/24/2023

Applicability

Dearborn, Troy