PROCEDURE
Corewell Health East - AmniSure Rupture of Fetal Membranes (ROM) - Dearborn & Troy

This Procedure is Applicable to the following Corewell Health sites:
Corewell Health Beaumont Troy Hospital, Corewell Health Dearborn Hospital

Applicability Limited to: N/A

Reference #: 33586

Version #: 2

Effective Date: 10/21/2025

Functional Area: Clinical Operations, Laboratory

Lab Department Area: Lab - Chemistry, Lab - Point of Care

1. Principle

A. This document describes how to perform AmniSure® ROM (AmniSure) testing and provides quality control (QC) and troubleshooting instructions for laboratory and non-laboratory personnel.

B. The AmniSure 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 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. Only a test line or no lines indicate an invalid test.

C. The AmniSure 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 rupture of membranes (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 cervical-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.

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

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

2. Responsibility

Personnel who have completed the competency requirements will perform this testing.

3. Specimen

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

B. Patient Preparation
   1. Patients should avoid disinfectant solutions or medications in the vagina six hours prior to specimen collection.

C. Patient Identification
   1. Refer to the Patient and People Identification Policy.

D. Specimen Labelling
   1. Bedside/Point of Care (POC) Testing (Dearborn): 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's name and identification (ID) number
   2. Laboratory Testing (Troy): A laboratory order must be placed in Epic. The specimen must be labelled with the laboratory specimen label prior to being transported away from the patient's bedside.

E. Specimen Collection, Handling, Transportation and Processing
   1. Verify that the specimen collection swab is not expired.
   2. Only the sterile polyester swab provided in the AmniSure kit should be used for specimen collection. Remove the swab from the package. The swab should not touch anything prior to inserting 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 one minute.
   5. After removing the swab from the vagina, immediately place the polyester tip into the labeled solvent vial and rotate the swab for one minute.
   6. Test the sample immediately after specimen collection (Dearborn) or send the solvent vial to the main laboratory (Troy) for testing. See the procedure section below for more information.
      a. If the swab is tested away from the patient's bedside, the solvent vial must be labelled with the patient's name and ID.
      b. If testing cannot be performed immediately or is sent to the laboratory for testing, the labeled solvent vial may be sealed and stored for four hours after collection at room temperature (20-25°C). The solvent vial may be stored refrigerated (2-8°C) up to six hours after collection. See the Procedure section below for steps on how to prepare the solvent vial.
      c. Troy: Place the sample in a biohazard bag prior to transit to the laboratory.

F. Specimen Rejection Criteria
   1. Specimens collected from a patient that used disinfectant solutions or medications in the vagina less than six 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

4. Reagent/Equipment Needed

A. Reagents
   1. AmniSure® ROM Test Kit (FMRT-1-25: 25 test kit or FMRT-1-10: 10 test kit)
      a. The kit contains the instructions for use, test strips stored in a foil pouch with desiccant, sterile polyester specimen collection swabs and test solvent vials.
         1) The solvent contains 0.9% sodium chloride, 0.01% Triton X-100 and 0.05% NaN_3
      b. Storage and Handling: Store the kits in a dry location at 4-25°C (40-77°F). Do not freeze.

c. Expiration: When stored in the foil pouch at the recommended temperature, the test is stable until the “Use By” date printed on the foil pouch.
d. Warnings and Precautions: For *in vitro* diagnostic use only. Do not interchange components from other kit lots.

2. AmniSure® ROM Test Positive Control
   a. The kit contains one vial of positive QC material and one solvent vial.
      1) The positive QC contains 10 ng of PAMG-1 protein purified from human amniotic fluid and lyophilized buffered saline.
      2) The solvent is a water-based solution containing distilled water, 0.98% sodium chloride, 0.01% Triton X and 0.05% NaN_3.
   b. Storage and Handling: Store in a dry place at 2-25°C. Do not freeze. Reconstitued controls can be refrigerated for up to 24 hours at 4-8°C.
   c. Expiration: Do not use beyond the expiration date printed on the vials.
   d. Warnings and Precautions: For *in vitro* diagnostic use only. Each control is a single-use disposable unit. Components cannot be reused.
3. AmniSure® ROM Test Negative Control
   a. The kit contains one vial of negative QC material and one solvent vial.
      1) The negative QC contains a sucrose solution.
      2) The solvent is a water-based solution containing distilled water, 0.98% sodium chloride, 0.01% Triton X and 0.05% NaN_3.
   b. Storage and Handling: Store in a dry place at 2-25°C.
   c. Expiration: Do not use beyond the expiration date printed on the vials.
   d. Warnings and Precautions: For *in vitro* diagnostic use only. Each control is a single-use disposable unit. Components cannot be reused.

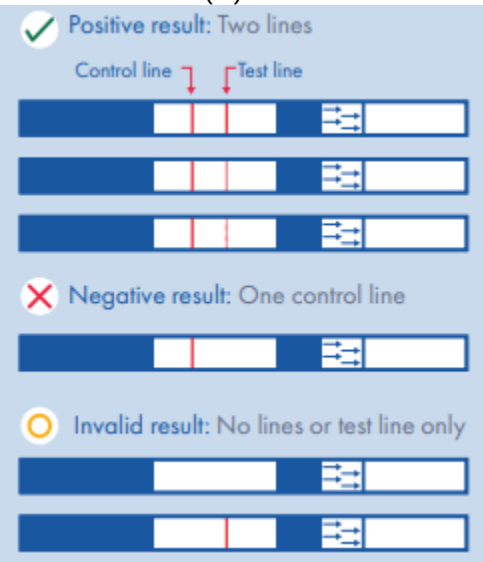
B. Equipment and Supplies
   1. Timer or Clock
   2. Sample Rack
   3. Conical Tube (For Transport from Bedside)
   4. Biohazard Bag (For Transport to Laboratory)

5. 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. Two levels of external controls are performed:
   1. With each new lot of test kits,
   2. Monthly when strips are stored for more than 30 days and
   3. To confirm test performance when test results do not correlate with the patient's clinical presentation.
C. Internal QC Procedure
   1. Internal QC is performed with each test to verify adequate sample volume, confirm capillary migration occurred, and proper procedural steps were followed.
      a. 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.
D. External QC Procedure
   1. Uncap the AmniSure solvent (liquid) vial.
   2. Uncap the positive control (freeze-dried) vial.
   3. Add the solvent (liquid) to the positive control (freeze-dried) vial.
      a. Note: The polyester swab is not needed for the external QC procedure.
   4. Cap the solution and mix by shaking vigorously for 30 seconds to allow for full reconstitution.
   5. Obtain an AmniSure test strip and open the foil pouch to remove the strip.
   6. Insert the white end of the test strip into the control solution.
      a. Note: The arrows on the test strip should be pointed down when introduced in the solution.

7. Allow the test strip to remain in the solution for five minutes.
    a. Do not read or interpret the results after 10 minutes have passed since first dipping the test strip into the vial.
8. Read the results by placing the test strip on a clean, dry, flat surface.
9. Document the external QC on the POC AmniSure Monthly QC / New Lot QC Validation form.
10. Repeat the External QC Procedure steps for the negative control.
11. Discard the test strips and vials in a biohazard or biohazard sharps container.

E. QC Result Interpretation
1. The control line and test line must be present for the positive external control (√).
2. Only the control line must be present for the negative external control (X).
3. The control line must be present for each patient and QC run. If the control line is not present, the test is invalid (O).



F. QC Failure Procedure
1. If one or one or more parameters are out of the acceptable range, appropriate corrective action must be taken and documented. Patient testing must be discontinued until the problem is resolved.
2. If unacceptable QC results appear, the following items should be verified:
    a. Verify that the QC and strips are not past the expiration dates.
    b. Confirm proper temperature storage for QC and strips.
    c. Observe the strip for defects (such as bending).
    d. Verify that the control/solvent solution was mixed well prior to testing.
    e. Confirm that the proper procedure and technique were followed.
    f. Repeat the test using new QC and/or new strips.
    g. If the above items do not resolve the issue, discontinue patient testing.
        1) Dearborn: Call 313-436-2367, 313-593-7970, 313-593-7592, or 313-982-5674.
        2) Troy: Notify the appropriate upline laboratory team member of the issue.

6. Procedure

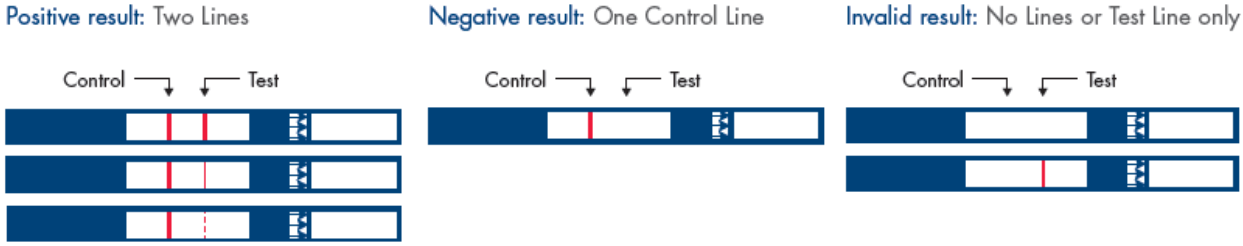
A. A provider test order, communication order or protocol criterion review must precede testing.
1. Troy: The specimen collection area must place a laboratory order in Epic: AmniSure Qualitative LAB4016.

B. Confirm that the supplies have not expired.
C. Remove a solvent vial from the kit and label it with the patient's name and ID.
D. Hold the solvent vial by the cap and shake well. Verify that all the liquid in the vial is at the bottom.
E. Open the solvent vial and place it into a rack to keep vertical.

F. Collect the patient sample as indicated in the Specimen Collection and Handling section above.
G. After removing the swab from the vagina, immediately place the polyester tip into the labeled solvent vial and rotate the swab for one minute.
H. Remove and dispose of the swab in a biohazard container or biohazard sharps container.
    1. Troy only: Package properly labeled solvent vial in a biohazard bag and send to the laboratory.
I. Tear open foil pouch at the tear notches and remove AmniSure test strip.
    1. Use the AmniSure test strip within six hours after removing it from the foil pouch.
J. Dip white end of the test strip (marked with arrows facing downward) into the vial with the solvent.
K. 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.
L. Remove the test strip from the vial.
M. Read the results by placing the strip on a clean, dry, flat surface in a well-lit environment using either natural light or fluorescent light.
    1. Do not read or interpret results after 15 minutes have passed since placing test strip into vial.
N. 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.
O. Discard the vial, swab and test strip in a biohazard container or biohazard sharps container.

7. Results/Interpretation

A. Interpretation of Results
    1. A positive result is indicated by two lines in the test region and is indicative of leakage of amniotic fluid and the fetal membrane rupture in patients.
    2. A negative result is indicated by the presence of a control line and absence of the test line.
    3. The presence of no lines or only a test line indicates an invalid test result. Do not interpret this as a negative result. Invalid tests must not be reported or acted upon clinically.



    4. 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.

B. Unexpected Results
    1. Negative AmniSure® results alone may not rule out membrane rupture. This test may be used in conjunction with other ROM tests such as Corewell Health East - pHizatest - All Beaumont Hospitals and Corewell Health East - Point of Care Fern Test - Dearborn, Farmington Hills, Trenton, Troy, Wayne, where available.
    2. Results that are questionable or are not consistent with the patient's clinical status should be repeated.

C. Result Reporting
    1. Dearborn: 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 personnel identification.
    2. Troy: Laboratory staff will document results in Epic Beaker.

D. Reportable Values
    1. Positive: Rupture
    2. Negative: No membrane rupture

E. System Downtime

1. If the electronic health record (EHR) or laboratory information system (LIS) is down, follow the department's downtime procedure and verify that the date/time of test, QC outcome, patient result and testing personnel are documented on the appropriate downtime log.

8. Limitations and Interfering Substances

A. Limitations
   1. A false negative test may result in an inadequate level of care for newborns less than 37 weeks gestation.
   2. False negative results can delay the diagnosis of rupture of membranes and can increase the risk of chorioamnionitis, oligohydramnios and fetal umbilical cord accident.
   3. Interrupted leakage with minimal residual fluid can lead to a false negative result.
   4. 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.
   5. Do not use damaged components of the test kit.
   6. Do not reuse the test kit components.
   7. Do not bend or fold the test strip or the aluminum foil pouch with the test strip in it.
   8. The AmniSure test results are qualitative. Make no quantitative interpretation based on the test results.
   9. 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.
   10. 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.
   11. Test performance in patients without signs or symptoms of ROM is unknown.
   12. Use results in conjunction with other clinical information.
   13. Failure to detect membrane rupture does not ensure the absence of membrane rupture.
   14. Patients may labor spontaneously despite a negative test result.
   15. Components from other AmniSure kits and/or lot numbers are not interchangeable.

B. Interfering Substances
   1. Vaginal infections and/or urine do not interfere with the results of the AmniSure test.
   2. Studies have shown that there is no interference of sperm.
   3. 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.
   4. The performance of the AmniSure test has not been established in the presence of meconium in the amniotic fluid.

9. 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.

10. Revisions

Corewell Health reserves the right to alter, amend, modify or eliminate this document at any time without prior written notice.

11. Resources

A. Corewell Health East - Point of Care - Downtime Result Recording, Reporting, and Recovery - All Beaumont Hospitals
B. Corewell Health East - Laboratory Downtime Procedure - All Beaumont Hospitals

12. References

A. AmniSure® ROM Test Instructions for Use, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1135629 Rev. 07 11/2024. www.qiagen.com/us.
B. AmniSure® ROM Test Positive Control, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1091028 Rev. 02 3/21/2017. www.qiagen.com/us.
C. AmniSure® ROM Test Negative Control, QIAGEN® 19300 Germantown Road, Germantown, MD 20874. 1091028 Rev. 02 3/21/2017. www.qiagen.com/us.

13. Procedure Development and Approval

Document Owner:
Laura Judd (Operations Specialist)

Writer(s):
Jessica Czinder (Point of Care Coordinator)

Reviewer(s):
Avani Shah (Medical Technologist Lead), Kristin Russell (Mgr, Laboratory), Laura Judd (Operations Specialist)

Approver:
Brittnie Berger (Dir Sr, Lab Operations), Caitlin Schein (Staff Physician), Christopher Ferguson (Dir, Laboratory Services), Helga Groat (Mgr, Laboratory), Jeremy Powers (Chief, Pathology), Masood Siddiqui (Staff Pathologist), Sarah Britton (VP, Laboratory Svcs), Steven Truscott (Tech Dir, Clin Chemistry, Path)

14. Keywords

AmniSure, ROM, PROM, rupture of membranes