Performing Rupture of Membrane Testing Using ROM Plus

Purpose

To provide instruction for performing the ROM Plus (Rupture of Fetal Membranes) test. The ROM Plus test is a rapid, qualitative test for the *in vitro* detection of amniotic fluid PP12 (Placental Protein 12, also known as Insulin-like Growth Factor Binding Protein-1/IGFBP-1) and AFP (Alpha-fetoprotein) proteins in vaginal secretions of pregnant women. The test should be used to aid in the detection of ROM in patients with clinical signs/symptoms suggestive of fetal membrane rupture.

Policy

- Internal Quality Control is performed with each specimen tested.
- Upon receipt of new shipment/lot #, immediately perform the following:
  a) Internal and External QC
  b) Kit crossover testing will be performed per each new shipment or lot # using previously tested external QC material
  c) External QC must be run every 30 days
  d) External QC must be run if the test kit has not been stored according to its labeling indications
- Patients presenting with reported signs, symptoms or complaints suggestive of rupture of membranes will be tested using the ROM Plus Rupture of Fetal Membranes (ROM) test.
- Order test code: FROM

Principle

The sample is collected by placing a swab on the vaginal mucosal lining for 15 seconds. The swab is then mixed into a vial containing buffer solution, and the diluted sample is applied to the sample pad of the test strip via the sample well on the cassette. The ROM Plus test strip is a lateral flow device. The sample substance flows from the pad region of the strip to the test region. During migration, the sample reacts with mono/polyclonal antibodies bound to the test strip membrane. These antibodies are immunoreactive to a combination of proteins, PP12 and AFP, which are markers of amniotic fluid. These two proteins were selected as a marker of fetal membranes rupture due to their unique characteristics, i.e. their high level in the amniotic fluid, low level in blood and extremely low background level in the cervicovaginal secretions when the fetal membranes are intact. The test result is indicated visually over the next 20 minutes by the presence of one or two lines. If the sample contains the PP12 and/or AFP markers of amniotic fluid, it binds to the antibody of the test line, causing the test line to appear and indicating a positive result. If the sample does not contain the PP12 and/or AFP specific to amniotic fluid, only the control line will be visible, indicating a negative result.

Continued on Next Page

Performing Rupture of Membrane Testing Using ROM Plus

Process

- ROM Plus test will be ordered STAT by Nursing personnel in HIS –order is Fluid Rupture of Membranes (FROM) & call the laboratory to obtain collection materials.
- The lab will respond by sending the collection kit which contains the swab and vial via pneumatic tube to the Family Birth Center.
- RN or physician will perform the collection and extraction steps, label the specimen and transport to the lab either via pneumatic tube system or hand carry the specimen to the lab, taking caution to prevent accidental leakage of the vial.
- Testing of the specimen will be performed by a CLS or MLT

Specimen Collection

The sample is obtained by an RN or physician using the following procedure.

1. Identify patient according to patient identification policy.
2. Follow steps below to collect sample:
   - Collect sample of vaginal secretions using the sterile vaginal swab provided in kit. (ROM Plus test does not require a speculum exam.)
   - Remove the contents of the collection kit from the packaging.
   - Holding the buffer vial in an upright position, remove the shipping cap and set the vial aside while maintaining an upright position to avoid spilling the buffer.
   - Remove the sterile swab from its package to collect a sample from the surface of the vagina. The tip of the swab should not touch anything prior to its insertion. Insert the swab tip into the vagina 2-3 inches (5-7 cm) deep. Withdraw the swab after a **minimum of 15 seconds**.
   - Place the swab tip into the vial. Mix the swab in the buffer. Break off the swab tip at the scored mark and leave the tip in the vial. Place the **shipping cap** back on the vial. Take caution to NOT place the drop dispenser lid on the vial.
   - Label vial with patient identification and date, time, initials of collection.
   - Transport to the lab either via pneumatic tube system or hand carry the specimen to the lab, taking caution to prevent accidental leakage of the vial.
   - NOTE: Significant amounts of blood discharge may cause the test to malfunction and is not recommended.

Continued on Next Page

Performing Rupture of Membrane Testing Using ROM Plus

Equipment/ Supplies

- External Timer
- ROM Plus Test Kit: ROM Plus test cassettes, each in foil pouch with desiccant, and ROM Plus Collection Kit (Sterile Polyester vaginal swabs and plastic vials with buffer solution)
  - Store in a dry place at 4-24°C (40-75°F)
  - Do not freeze
  - When stored in the foil pouch at the recommended temperature, the test is stable until the Expiration Date on the pouch
  - Use ROM Plus within six hours after opening the foil pouch
  - Use ROM Plus within six hours of collecting the vaginal swab sample and placing it into the buffer vial
- ROM Plus Quality Control Kit
  - Lyophilized positive and negative controls
  - Store in a dry place at room temperature to the expiration date
  - Note: The positive control is obtained from purification of human amniotic fluid, assayed to provide the appropriate concentration of PP12 and AFP, and then lyophilized. Buffer is mixed with the positive sample from an integrated sealed glass ampoule when performing testing. The negative control is stabilized solvent containing normal saline but does not contain PP12 or AFP.

Quality Control

Internal QC

- Each ROM Plus test has 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. If no lines are visible, the test result is invalid and should be repeated.
- The appearance of the control line (C) assures that adequate sample volume was present and that adequate capillary migration (lateral flow) of the sample has occurred.

Continued on Next Page

Performing Rupture of Membrane Testing Using ROM Plus

External QC Follow the steps below to perform the external QC testing.

Step	Action
1.	Prepare two ROM Plus test cassettes, label one for the Positive Control and one for the Negative Control. Note: Since this is a quality control test and no human sample is required, the polyester vaginal swab and standard dropper vial should not be used.
2.	Tear open the Positive Control Vial foil pouch and remove the vial. Gently bend or squeeze the Positive Control vial, breaking the glass ampoule inside. **Mix the buffer with the lyophilized positive sample for at least 45 seconds**. Be careful not to let the sample drip out of the vial.
3.	Gently bend or squeeze the Negative Control Vial, breaking the glass ampoule inside. Be careful not to let the sample drip out of the vial.
4.	Add 4 drops of the Positive Control solution to one ROM Plus test cassette. Add 4 drops of the Negative Control solution to the other ROM Plus test cassette.
5.	Start a timer for 20 minutes. Note: DO NOT USE THE CASSETTE INTERNAL TIMER.
6.	Read the test result at 20 minutes – refer to Results Interpretation – and record results on appropriate QC log.
7.	If external QC performs as expected, proceed with patient testing. If external QC does not perform as expected, repeat testing using new control vial. If repeat testing does not perform as expected, do not proceed with patient testing. Contact Clinical Innovations at (888)-268-6222.

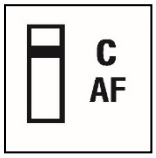
Continued on Next Page

Performing Rupture of Membrane Testing Using ROM Plus

Procedure Follow the steps to perform the ROM Plus test.

Step	Action
1.	Obtain appropriate patient testing log.
2.	Record kit lot # and expiration date at top of log.
3.	Tear open foil pouch at the tear notches and remove ROM Plus cassette.
4.	Remove the shipping cap and place the drop dispenser lid on the buffer vial.
5.	Add 4 drops of the sample/buffer solution to the sample well of the cassette.
6.	Start a timer for 20 minutes. Note: DO NOT USE THE CASSETTE INTERNAL TIMER.
7.	Wait 20 minutes for test results to manifest in the test window (C/AF), unless 2 lines are clearly visible. A positive test result may be visible early (within 1-3 minutes) or may take the full 20 minutes.
8.	Record test result and result of the internal control on the log.

Interpretation

Observation	Result
If only a control line (C) is present 	Negative for Membrane Rupture
If both the control line (C) and test line (AF) are present 	Positive for Membrane Rupture
No Lines Present in Test Area or just the test line (AF) is visible 	Invalid. Request new sample to be submitted for testing.

- Do not interpret test results based on darkness of the stripes. Darkness of the stripes may vary, however the test is valid even if the stripes are faint.
- A light visible line located in the test (AF) region should be considered a positive; in addition, very high concentrations of proteins may result in light test (AF) line.

Continued on Next Page

Performing Rupture of Membrane Testing Using ROM Plus

Reporting Follow the steps to result patient results in LIS.

Step	Action
1.	At Sunquest function prompt, enter MEM.
2.	At Worksheet prompt, enter RVFLDM.
3.	Enter "A" to proceed to the requested worksheet.
4.	Enter the Accession number to be resulted at the Accession prompt.
5.	Using the table below, enter the appropriate response. **Test Result** / **Report** Positive for Membrane Rupture / POS Negative for Membrane Rupture / NEG Invalid / *Do Not Report*- Cancel test using code RNNOT (Nursing unit notified of need to recollect specimen) **NOTE:** The ETC "ROMI" will auto-append to results. ROMI = The test may report false results in patients with intact membranes and therefore decisions to induce labor should not be based solely on the ROM Plus test results.
6.	Enter "A" to accept and save results.
7.	Verify correct manual entry of results. (Refer to "Detecting and Correcting Data Entry Errors" LG.POST07.00-SS)

Procedure Notes

- The test may report positive results in patients with intact membranes and therefore decisions to induce labor should not be based solely on the ROM Plus test results.
- ROM diagnoses should not be based on any single test.
- ROM Plus is for in vitro diagnostic use only.
- Allow pouch containing ROM Plus to reach room temperature prior to test.
- Each ROM Plus kit is single use and disposable and should not be reused.
- ROM Plus results are qualitative. No quantitative interpretations should be made.
- Elevated fetal serum, urine, cord blood, and amniotic fluid as well as maternal serum levels of AFP have been reported in the literature in various developmental disorders such as neural-tube defects, hypothyroidism, autoimmune states, congenital heart defects, cystic fibrosis, etc. ROM Plus has not been evaluated for potential interference in these conditions.

Continued on Next Page

Performing Rupture of Membrane Testing Using ROM Plus

Interference

- ROM Plus test kits will function properly with trace amounts of blood in the sample. Significant amounts of blood discharge may cause the test to malfunction and is not recommended.
- To determine interference and cross-reactivity of the assay, Tylenol, aspirin, KY Gel, and three different bath products (Lever soap, Noxzema Cream, Pert Shampoo) were spiked into the low positive control at a final concentration of 0.1% without visual loss of activity. The same bath products were spiked into the negative-matrix control and shown to be negative.
- In addition, human semen, urine and blood were spiked into the low positive at a 10% final concentration without loss of activity. Human semen, urine, blood were also spiked into the negative control matrix and shown to be negative.
- The PP12/IGFBP-1 assay does not cross react with IGFBP-2, IGFBP-3, and IGFBP-4 based on Western Blot results.
- ROM Plus was shown to be negative when tested with specimens that were positive for bacterial vaginoisis and other sexually transmitted diseases.

Supporting Documents

- Form A: Patient Testing & Internal QC Results Log – ROM Plus
- Form B: ROM Plus Quality Control Log

References

- ROM Plus test package insert, Clinical Innovations, Rev D 2016
- ROM Plus Quality Control Kit package insert, Clinical Innovations, Rev B
- Rutanen et al., Radioimmunoassay of placental protein: levels in amniotic fluid, cord blood and serum of healthy adults, pregnant women and patients with troblastic disease. AM J Obstet Gynecol. 1982; 144:460
- Seppala M. Ruoslahti E: Alpha-fetoprotein in amniotic fluid as an index of gestational age. AM J Ostet Gynecol. 1972; 114:595
- Thomasino T, Levi C. Diagnosing Rupture of Membranes Using Combination Monoclonal/Polyclonal Immunologic Protein Detection. J Reproductive Medicine. May-June 58 (2013): 187-194

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