Methodist Health Services Corporation
& UnityPoint Health Methodist
Department of Pathology
Peoria, IL 61636

Effective Date: 1/19/17
New Procedure

ROM Plus
Fetal Membranes Rupture Test
Qualitative Immunochromatrographic
Lateral flow device

I. PRINCIPLE

The ROM Plus® (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. ROM Plus® detects PP12 (also known as IGFBP-1) and AFP protein markers of amniotic fluid in vaginal secretions. The test is for use by health care professionals to aid in the detection of ROM in pregnant women when patients report signs, symptoms or complaints suggestive of ROM.

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. The test result is indicated visually over the next 5-20 minutes by the presence of one or two lines. One line indicates no membranes ruptured, two lines indicates there is a rupture.

The ROM Plus® Test uses the principles of immunochromatography to detect human PP12 (Placental Protein 12, also known as Insulin-like Growth Factor Binding Protein-1/IGFBP-1) and AFP (Alpha-fetoprotein) proteins present in amniotic fluid of pregnant women. 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 cervicovaginal secretions when the fetal membranes are intact.

During the test procedure, the sample reacts with mono/polyclonal antibodies bound to the test strip membrane. These antibodies are immune-reactive to a combination of proteins, PP12 and AFP, which are markers of amniotic fluid. As the membrane absorbs the liquid sample, a control line will appear, indicating a sample was properly applied. 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.

II. CLINICAL SIGNIFICANCE

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.

III. SPECIMEN

The test does not require speculum examination that is used routinely today for ROM diagnosis. A sample of amniotic fluid (taken by vaginal swab) is placed into a vial with a buffer solution.

Sample from the surface of the vagina. Use the sterile polyester swab provided.

Use ROM Plus® within six (6) hours of collecting the vaginal swab sample and placing it into the buffer vial.

Presence of significant blood, collected with the swab, can lead to false positive results and specimen will be rejected.

IV. REAGENTS

Materials Provided: ROM Plus® test cassettes (lateral flow devices), each in foil pouch with desiccant. Each Test cassette contains:

1. ROM Plus® test strips (lateral flow devices), each in foil pouch with desiccant
2. Sterile Polyester vaginal swabs.
3. Plastic vials with buffer solution.

Store the ROM Plus® kit in a dry place at room temperature. 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 (6) hours after opening foil pouch.

V. INSTRUMENTATION/EQUIPMENT - See Reagents.

VI. CALIBRATION - NA

VII. QUALITY CONTROL

A. INTERNAL CONTROL:

1. 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.

2. 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.

B. EXTERNAL CONTROL:

External QC will be completed for each new lot number or shipment of test material, monthly, or if there is suspicion of improper storage.

1. The positive control is obtained from purification of human amniotic fluid, assayed to provide the appropriate concentration of PP12 and AFP and then lyophilized.
2. Buffer is mixed with the positive sample from an integrated sealed glass ampoule when performing testing. The buffer and lyophilized control form a stabilizyme solvent containing normal saline (same salinity as amniotic fluid), a pH of 7.4 (same as amniotic fluid) and a total protein concentration of 4-5 mg/ml, 20 ng/ml PP12 and 600 ng/ml AFP after dilution with contained ampoule buffer.
3. The preservative Stabilizyme in the buffer ampoule contains sucrose 5% BSA stabilizer and bovine serum albumin to provide 4-5mg/ml protein concentration along with preservative 0.1% Proclin 950 at a pH of 8.1 (similar to amniotic fluid).
4. The negative control is a stabilized solvent containing normal saline (same salinity as amniotic fluid), a pH of 7.4 (same as amniotic fluid), but does not contain PP12 or AFP.
5. The lyophilized positive and negative controls can be stored in a dry place at room temperature until the expiration date.

Quality Control Procedure

1. External Positive Control: lyophilized human amniotic fluid (self contained glass ampoule of buffer)

2. External Negative Control: Saline solution (self contained glass ampoule of buffer).

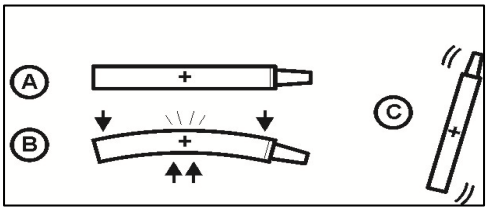
3. Prepare two ROM Plus® test cassettes, label one for the Positive Control and one for the Negative Control. Since this is a quality control test and no human sample is required, the polyester vaginal swab and standard dropper vial should not be used.

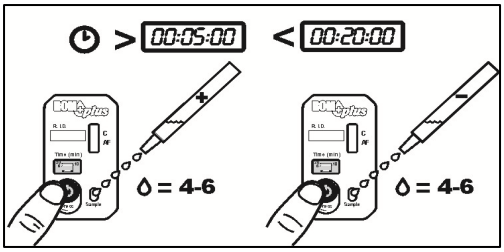
4. Tear open the Positive Control Vial foil pouch and remove the vial. Gently bend or squeeze the Positive Control vial, breaking the glass ampoule inside. Mix the buffer with the lyophilized positive sample until it is completely dissolved. Be careful not to let the sample drip out of the vial.

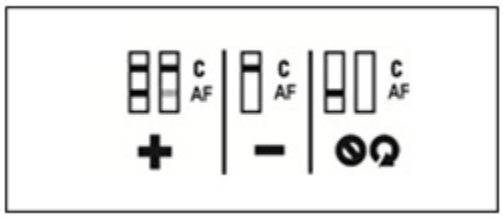
5. Gently bend or squeeze the Negative Control vial, breaking the glass ampoule inside. Be careful not to let the sample drip out of the vial.

6. Add 4-6 or more drops of the Positive Control solution to one ROM Plus® test cassette. Add 4-6 or more drops of the Negative Control solution to the other ROM Plus® cassette. Set a timer for 20 minutes. Start the timer. Wait 20 minutes for test results to manifest in the test window (C/AF).

7. Read the test results at 20 minutes.







VIII. PROCEDURE:

A. To be performed in Labor and Delivery by competent staff:

1. Remove the ROM Plus® contents from the packaging. Holding the buffer vial in an upright position, remove the shipping cap and set the vial aside.

2. 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 (5-7cm) deep. Withdraw the swab after a minimum of 15 seconds.

3. Place the swab tip into the vial and mix the swab in the buffer solution for at least 15 seconds. Break off the swab tip at the scored mark and leave the tip in the vial.

4. Place the drop dispenser lid on the buffer vial and dispose of the remaining swab stick. Send specimen to the laboratory.

B. To be performed in the Laboratory:

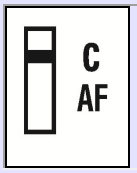
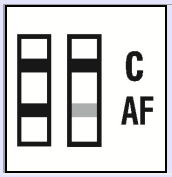
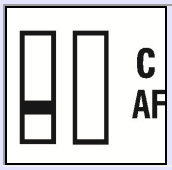
1. Tear open the foil pouch and remove the ROM Plus® cassette. Label the cassette with the LIS label and add at least 4-6 drops of the sample/buffer solution to the sample well of the cassette.

2. Start the timer by firmly pressing and rolling thumb over the button from left to right. Cassette timer does not have an audible sound so start a timer with an audible beep to remind you to read the test.

3. Wait 20 minutes for test results to manifest in test window (C/AF). Darkness of the stripes may vary, however the test is valid even if the stripes are faint. Do not interpret test results based on darkness of the stripes.

4. Read the test result at 20 minutes. If only a control line (C) is visible, the test result is negative. If both the control line (C) and test line (AF) are visible, the test result is positive. If no lines are visible, or just the test line (AF) is visible, the test result is invalid. 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 a light test (AF) line. It is recommended to read the strip by 20 minutes.

C. Reporting Results

If only a control line (C) is seen: the test is **negative**, no membrane rupture.	
If both the control line (C) and test line (AF) are seen: the test is **positive**, membranes are ruptured.	
If no lines are visible, or just the test line (AF) is visible, the test result is **invalid** and should be repeated.	

1. All ROM Plus® patient test results should be documented in the LIS as well as the internal QC result. Document the kit lot # and expiration date along with QC/OK comment.

IX. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. ROM diagnoses should not be based on any single test.

B. ROM Plus® is for in vitro diagnostic use only.

C. ROM Plus® is for healthcare professionals use only.

D. All instruction should be followed carefully for accurate results.

E. Each ROM Plus® test kit is single use and disposable and should not be reused.

F. ROM Plus® results are qualitative. No quantitative interpretations should be made.

G. ROM Plus® test kits will function properly with trace amounts of blood in the sample. Significant amounts of bloody discharge may cause false positive results and specimen should be recollected.

H. Safety precautions should be observed when collecting, handling, and disposing of test samples. Used test kits are bioharzardous.

I. 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.

J. The test may report positive results in patients with intact membranes (see specificity in the performance section) and therefore decisions to induce labor should not be based solely on the ROM Plus® test result.

X. LIMITS OF THE TEST

A. The ROM Plus® [fetal] Membranes Rupture Test is for the in vitro detection of human amniotic fluid PP12 and AFP proteins in vaginal secretion of pregnant woman. The test should be used to evaluate patients with clinical signs/symptoms suggestive of fetal membranes rupture.

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

C. Results should be used in conjunction with other clinical information.

D. Bleeding, placenta Previa, and performing digital exams prior to sample collection can lead to inaccurate test results.

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

F. Women may labor spontaneously despite a negative test result.

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

H. Reasons for test failure:

a. Failure to follow test instructions

b. Improper storage of test kit (frozen or above room temperature).

XI. INTERFERENCE

A. 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.

B. 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.

C. The PP12/IGFBP-1 assay does not cross react with IGFBP-2, IGFBP-3, and IGFBP-4 based on Western Blot results.

D. ROM Plus® was shown to be negative when tested with specimens that were positive for bacterial vaginosis and other sexually transmitted diseases.

XII. REFERENCES

A. 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.

B. Seppala M. Ruoslahti E: Alpha-fetoprotein in amniotic fluid as an index of gestational age. Am J Obstet Gynecol. 1972; 114:595.

C. ROM Plus® test package insert

D. Manufacture-recommended QC Reference Sheet and Instructions for Use

MMCI Laboratory is a CAP accredited facility, as of 7/1/11 the responsibility of new and/or substantially revised policies and procedures will be restricted the Laboratory Director whose name appears on the CLIA certificate, whose signature appears below. The biennial review will be completed by the Administrative Director.

Policy Created by: Kim Paige Date: January 19, 2017

Medical Director Approval: Elizabeth A. Bauerlein MD **Date: January 22, 2017**

CHANGE OF/TO SECTION MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
1/20/2017	Katherine Kasper, M.D.	Katherine A. Kasper MD

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Initial Release	Kim Paige	1/19/17

Reviewed by

LEAD	DATE	COORDINATOR/ MANAGER	DATE	MEDICAL DIRECTOR	DATE
K. Paige	1/19/17	Jane Bambenek	1/20/17	Katherine A. Kasper MD	1/20/17