PRINCIPLE:

The ROM Plus ® (Rupture Of [fetal] Membranes) Test is a rapid, non-instrumented, qualitative immunochomatographic test for the in vitro detection of amniotic fluid in vaginal secretions of pregnant women. 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 ruptures, 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 (Alphafetoprotein) 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. 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 Standard Operating Procedure Lab-8320 |Rupture of Membranes (ROM+) Page 2 of 7 inappropriate interventions (e.g., hospitalization of 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. The ROM Plus® test kit is a self-contained test system providing qualitative results that are both accurate and do not require collection methods such as speculum examination. SPECIMEN: Patient Preparation: N/A 1. Unit will collect a sample from the surface of the vagina. The test does not require speculu

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SPECIMEN: Patient Preparation: N/A

1. Unit will collect a sample from the surface of the vagina. The test does not require

2. For Collection Requirements, Go to Lab Web Site, Specimen Collection Resources & Forms.

3. The sample of amniotic fluid (taken by vaginal swab) is placed into a vial with a buffer solution. The swab is broken off at the score line and the vial with swab is sent to laboratory.

4. Label specimen as described in Lab-1330 Patient Identification.

5. The sample is stable for 6 hours after collection before testing.

Unacceptable Specimens:

1. Specimens received into the lab >6 hours after collection.

2. Un-labelled or inadequately labelled specimens.

3. Grossly bloody specimens.

4. Specimens not collected in the appropriate vial or specimens without the swab.

REAGENTS/MATERIALS:

1. ROM Plus Collection Kit - includes a polyester sterile swab, vial with buffer solution and the shipping cap for the vial. a. Store in a dry place at 4° to 24°C (40° to 75°F). Do not freeze.

2. ROM Plus® test cassettes (lateral flow devices), each in foil pouch with desiccant.

a. Store in a dry place at 4° to 24°C (40° to 75°F). Do not freeze.

b. When stored in the foil pouch at the recommended temperature, the test is stable until the “Expiration date” on the pouch.

c. Use ROM Plus® within six (6) hours after opening foil pouch. d. Use ROM Plus® within six (6) hours of collecting the vaginal swab sample and placing it into the buffer vial.

3. Quality Control ROM Plus kit contains one Positive and one Negative control vial.

a. External Positive Control: lyophilized human amniotic fluid assayed to provide the appropriate concentration PP12 and AFP. Buffer is mixed with the positive sample from an integrated sealed glass ampoule when performing testing. i. 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- 5mg/ml, 20ng/ml PP12 and 600ng/ml AFP after dilution with contained ampoule buffer.

ii. The preservative Stabilizyme in the buffer ampoule contains sucrose 5% BSA stabilizer and bovine serum albumin to provide 4-5mg/ml protein concentration

EQUIPMENT/INSTRUMENTATION: N/A

CALIBRATION: N/A

QUALITY CONTROL:

1. External Controls will be run:

a. Monthly. Controls will auto-order every 28 days.

b. Upon receipt of a new lot number of test kits.

c. If the laboratory temperature falls outside of acceptable ranges.

d. If the patient result is in question.

e. Document all control results in LIS. Indicate in yellow comment box, the cassette lot# and reason external controls were performed.

2. Internal Procedural controls are run with each test performed. Document in LIS.

a. A clear background on the device is the negative internal control.

b. A visible line in the Control area of the device is the positive internal control.

c. If there is no visible line in the control area, the test result is invalid and should be repeated.

d. If problems persist, contact the technical leader or the vendor. PERFORMANCEMedical Inc 2512 Northland Drive Saint Paul, Minnesota 55120 800-798-1631

3. Quality Assurance is monitored by participation in approved proficiency surveys.

4. Quality Control will be reviewed monthly by the Technical Leader or designated person.

PROCEDURE:

1. Tear open the foil pouch and remove the ROM Plus® cassette. Label cassette with unique identifier. Standard Operating Procedure Lab-8320 |Rupture of Membranes (ROM+)

2. Remove shipping cap and put drop dispenser on specimen vial. (Vaginal swab can remain in vial).

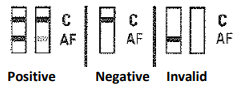
3. Add at least 4-6 drops of the sample/buffer solution to the sample well of the cassette.

4. Start the timer by firmly pressing and rolling thumb over the button from left to right.

5. Wait 5-20 minutes for test results to manifest in test window (C/AF).

A positive test result may be visible early (within 1-3 minutes) or may take the full 20 minutes. 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.

6. Read the test result. It is recommended to read the strip by 20 minutes.



MAINTENANCE: N/A

PROCEDURE NOTES:

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

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

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

4. Allow pouch containing ROM Plus® to reach room temperature prior to testing.

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

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

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

8. 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.

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

10. 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.

11. 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.

EXPECTED VALUES:

No rupture of membranes when test is negative.

Rupture of membrane when test is positive.

CALCULATIONS: N/A

INTERPRETATION: If only a control line (C) is seen: the test is negative, no membrane rupture. Standard Operating Procedure Lab-8320 |Rupture of Membranes (ROM+) Page 6 of 7 If both the control line (C) and the test line (AF) are seen: the test is positive, membranes are ruptured. 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. If no lines are visible, or just the test line (AF) is visible, the test result is invalid and should be repeated. If problems persist, contact technical leader and/or vendor (Performance Medical Inc. 800-798-1631).

LIMITATIONS:

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

2. You must follow all directions carefully to get an accurate reading of the results,

3. Presence of significant blood, collected with the swab, can lead to false positive results. In cases of only trace amounts of blood on the swab the test functions properly.

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

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

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

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

8. False negative results and delay in the diagnosis of rupture of membranes can increase the risk of chorioamnionitis, oligohydramnios and fetal umbilical cord accident. 9. Reasons for a test failure: a. Failure to follow test instructions b. Improper storage of test kit Interferences: 1. To determine interference and cross-reactivity of the assay, Tylenol, aspirin, KY Gel, and three different bath products (Lever Soap, Noxema 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. 2. 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 and blood were also spiked into the negative control matrix and shown to be negative. 3. The PP12/IGFBP-1 assay does not cross react with IGFBP-2, IGFBP-3 and IGFBP-4 based on Western Blot results. 4. ROM Plus® was shown to be negative when tested with specimens that were positive for bacterial vaginosis and other sexually transmitted diseases.

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3. ROM Plus® test package insert

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

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