

ROM+plus[®]

Fetal Membranes Rupture Test

INSTRUCTIONS FOR USE

INTENDED USE

The Clinical Innovations ROM Plus fetal membrane rupture test is a rapid, qualitative immunochromatographic test for the in vitro detection of amniotic fluid in vaginal secretions of pregnant women with signs and symptoms of ROM. The test detects AFP (alpha-fetoprotein) and PP12 (placental protein 12 or insulin growth factor binding protein) from amniotic fluid in vaginal secretion. The test is for prescription use by health care professionals to aid in the detection of rupture of membranes (ROM) in pregnant women in conjunction with other signs and symptoms.

REAGENTS AND COMPONENTS

Each ROM Plus kit contains:

- One ROM Plus test cassette with timer
- One sterile polyester vaginal swab
- One vial with buffer solution (phosphate-buffered saline)
- Directions for use (on each pouch)
- The cassette test strip contains the following antibodies: sheep antihuman PP12, goat antihuman AFP, mouse conjugated colloid gold antihuman PP12 & AFP.

TEST SUMMARY

ROM Plus is a self-contained test kit that provides qualitative results for rupture of fetal membranes and is performed at point-of-care sites. A speculum is not needed to obtain ROM Plus results. The test is non-invasive, with only a simple vaginal swab sample required.

The sample is collected by placing the swab on the vaginal mucosal lining for 15 seconds. The swab is then mixed into a vial containing 400 µL of buffer solution, and the diluted sample is applied to the sample pad of the test strip via the sample well on the cassette. A built-in timer is then activated and visualized as a convenient feature to indicate the time of the test. The liquid moves chromatographically and unidirectionally towards the absorbent pad.

TEST PRINCIPLE

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. As the membrane absorbs the liquid sample, a control line will appear, indicating a sample was 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.

STORAGE AND STABILITY

- Store the kit in a dry place at 4° to 24°C (40° to 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 (6) hours after opening foil pouch.
- Use ROM Plus within six (6) hours of collecting the vaginal swab sample and placing it into the buffer vial.

PRECAUTIONS, LIMITS AND WARNINGS

- ROM diagnoses should not be based on any single test.
- ROM Plus is for in vitro diagnostic use only.
- ROM Plus is for healthcare professional use only.
- Allow pouch containing ROM Plus to reach room temperature prior to test.
- All instructions should be followed carefully for accurate results.
- Each ROM Plus test kit is single use and disposable and should not be reused.
- ROM Plus results are qualitative. No quantitative interpretations should be made.
- 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.
- Safety precautions should be observed when collecting, handling, and disposing of test samples. Used test kits are biohazardous.
- 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.

Warning: 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 results.

METHOD OR PERFORMANCE

The ROM Plus assay was validated for the parameters of linearity, limit of detection, accuracy/reproducibility, sensitivity, specificity and cross-reactivity:

- High Concentration ("High Dose Hook" effect) - for the ROM Plus upper-detection range, the PP12 and AFP were tested. Concentrations of PP12 were tested up to 400,000 ng/ml and AFP up to 200,000 ng/ml with a positive visual results. (Although lines may be lighter in presence of very high AFP and PP12 concentrations, any line is considered positive.)
- For 100% of ROM Plus tests sampled, the lowest limit of detection (LOD) is 5 ng/ml for PP12, and 150 ng/ml for AFP. (These LOD concentrations refer to those in samples of vaginal secretions before dilution with buffer.)
- Reproducibility was tested on different days at six levels of amniotic fluid spiked into a negative control. The assay was run on three lots of ROM Plus to determine the visual positive or negative results. Two low positives, two moderate positives and two high positives were run on three lots of ROM Plus on four different days. No difference in activity was observed.
- To determine interference and cross-reactivity of the assay, Tylenol, aspirin, 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. 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. PP12 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 vaginosis and sexually transmitted diseases. All samples were pH > 4.5.

A multi-center prospective observational study was performed. Women included in the study were healthy pregnant women, 18 years of age or older, between 15-42 weeks of gestation reporting signs or symptoms of rupture of membranes (<34 ega [estimated gestational age], n=45; 34 to 24 ega, n=33; <24 ega, n=12). Patients with known placenta previa and active vaginal bleeding were excluded from the study. Initial evaluation included both the standard clinical assessment (Clin-Assess) for rupture of membranes comprising conventional clinical testing diagnosed by fluid leaking from the cervical os, or two of the following: pooling, positive nitrazine test, or ferning (SSE), as well as the new combination immunoassay ROM Plus containing the monoclonal and polyclonal antibodies to Placental Protein 12 (PP12) and Alpha-fetoprotein (AFP).

Clinicians performing the SSE were blinded from the ROM Plus results. Clinicians performing ROM Plus testing were masked from Clin-Assess results. The clinical standard of pooling/ferning/nitrazine which has been shown in the literature to have sensitivities and specificities of 51-97% and 16-90% respectively^{1,13} (when these tests are used individually) are not an ideal reference standard.^{12,16} However, it was used as the reference standard in this study understanding its limitations and because it is commonly used in clinical hospital protocol to evaluate ROM. Corrections for ROM based on subsequent patient chart review was not used in this study as it was in other immunological studies^{4,5} for the detection of ruptured membranes.

Of the 264 patients in the study, rupture of membranes from Clin-Assess (SSE) as the standard compared to ROM Plus results were: sensitivity of 99%, specificity of 75%, positive predictive value of 85%, and negative predictive value of 99%. Table 1 shows 95% confidence levels for different gestational ages. Of the false positives 23 out of 28 (82%) were term and 5 of 28 (18%) were < 37 weeks gestation indicating a large majority of the FP's were at term (≥ 37). Results in this study for pre-term were: sensitivity of 100%, specificity of 85%, positive predictive value of 85%, and negative predictive value of 100%. Studies as reported in the literature³ for a predicate device using uncorrected chart review data shows substantial equivalence to ROM Plus for the detection of ruptured membranes. One author has hypothesized that false positives noted later in pregnancy and near delivery may be related to amniotic membranes beginning to leak (or becoming more permeable).¹⁷

Table 1: Clinical Study - ROM Plus vs. Clinical Assessment (pooling/ferning/nitrazine)

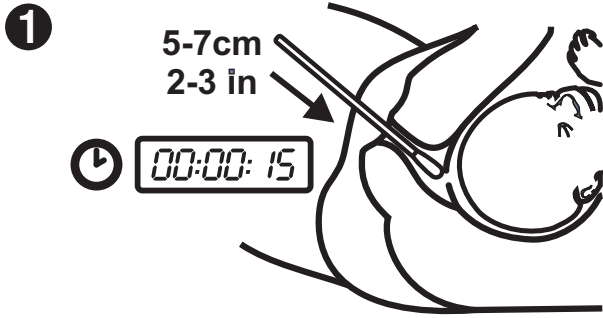
Combined	Clin-Assess	positive		negative		≥ 37	Clin-Assess	positive		negative	
		positive	negative	positive	negative			positive	negative	positive	negative
ROM Plus	positive	153	28	ROM Plus	positive	125	23				
	negative	1	82		negative	1	33				
Total: 264				Total: 182							
Sens 0.99 CI: 0.96 to 1.00				Sens 0.99 CI: 0.96 to 1.00							
Spec 0.75 CI: 0.66 to 0.82				Spec 0.58 CI: 0.46 to 0.71							
PPV 0.85 CI: 0.79 to 0.90				PPV 0.84 CI: 0.78 to 0.89							
NPV 0.99 CI: 0.94 to 1.00				NPV 0.97 CI: 0.85 to 0.99							
34-37 EGA	Clin-Assess	positive		negative		< 34	Clin-Assess	positive		negative	
		positive	negative	positive	negative			positive	negative		
ROM Plus	positive	18	3	ROM Plus	positive	10	2				
	negative	0	16		negative	0	33				
Total: 37				Total: 45							
Sens 1.00 CI: 0.82 to 1.00				Sens 1.00 CI: 0.72 to 1.00							
Spec 0.85 CI: 0.64 to 0.95				Spec 0.94 CI: 0.81 to 0.98							
PPV 0.87 CI: 0.65 to 0.95				PPV 0.83 CI: 0.55 to 0.95							
NPV 1.00 CI: 0.81 to 1.00				NPV 1.00 CI: 0.90 to 1.00							

QUALITY CONTROL

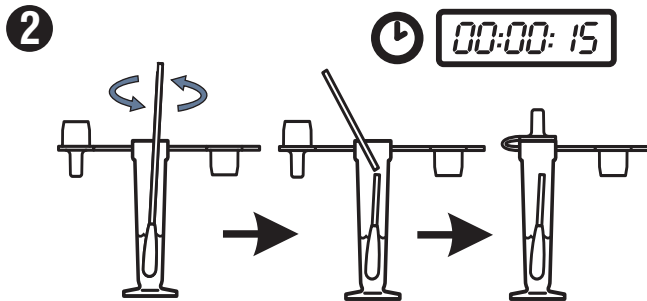
ROM Plus has control and test lines integral on each cassette test strip. The appearance of the control line helps ensure that the sample was properly applied to the test strip. The appearance of both control and test lines indicates proper use of the cassette. If no lines are visible, the test result is invalid and should be repeated.

ROM Plus Fetal Membrane Rupture Test Quality Control kit for external controls are available from Clinical Innovations, LLC. and are intended to monitor the performance of Clinical Innovation's ROM Plus fetal membrane rupture test cassette. The control test kits contain human PP12 and human AFP amniotic fluid proteins at concentrations of 20 ng/ml and 600 ng/ml vaginal secretion equivalence respectively. External negative controls that do not contain these proteins are also available in the Quality Control Kit. Always follow federal, state and local guidelines for quality control documentation.

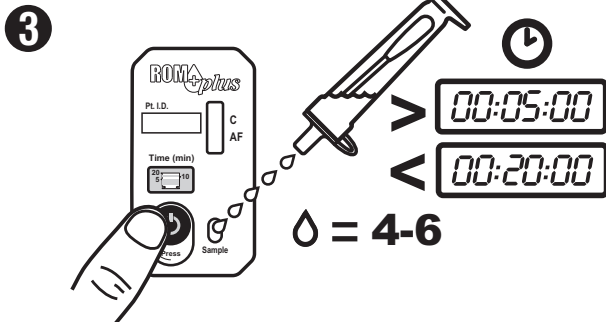
TEST PROCEDURE



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

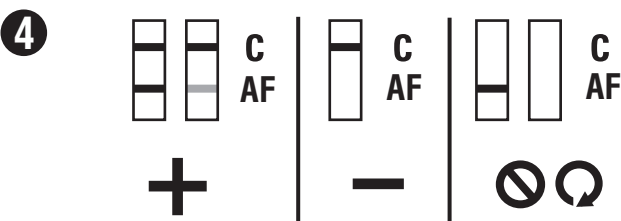


2. Place the swab tip into the vial. Mix the swab in the buffer. Break off the swab tip at the scored mark and leave the tip in the vial. Place the drop dispenser lid on the buffer vial and dispose of the remaining swab stick. Allow at least 15 seconds for the swab to be in the buffer solution.



3. Tear open the foil pouch and remove the ROM Plus cassette. Add 4-6 or more drops of the sample/buffer solution to the sample well (Sample) of the cassette. Start the timer by firmly pressing and rolling thumb over the timer button from left to right.

Wait 5-20 minutes for test result 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. The test is valid even if the stripes are faint. Do not interpret test result based on darkness of the stripes.



4. Read the test result. 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 and should be repeated. A light visible line located in the test (AF) region should be considered 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.

EXPECTED RANGES

IGFBP-1 concentration in amniotic fluid as determined in literature studies in amniotic fluid is between 10,500 and 350,000 ng/ml¹⁷ and for AFP from 2,800 to 26,000 ng/ml¹⁸ and in serum from 55 to 242 U/ml (equivalent to 33 to 290 ng/ml). Concentrations of IGFBP-12 (PP12) in amniotic fluid observed PP12 to be 100 to 1000 times higher than that in maternal serum.¹⁹ These studies also showed that concentrations of these proteins in urine to be negligible.¹⁷⁻¹⁹

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