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REVIEW

ROM Plus®: accurate point-of-care detection of ruptured fetal membranes

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Abstract: Accurate and timely diagnosis of rupture of fetal membranes is imperative to inform and guide gestational age-specific interventions to optimize perinatal outcomes and reduce the risk of serious complications, including preterm delivery and infections. The ROM Plus is a rapid, point-of-care, qualitative immunochromatographic diagnostic test that uses a unique monoclonal/polyclonal antibody approach to detect two different proteins found in amniotic fluid at high concentrations: alpha-fetoprotein and insulin-like growth factor binding protein-1. Clinical study results have uniformly demonstrated high diagnostic accuracy and performance characteristics with this point-of-care test that exceeds conventional clinical testing with external laboratory evaluation. The description, indications for use, procedural steps, and laboratory and clinical characterization of this assay are presented in this article.

Keywords: ROM Plus®, premature rupture of membranes, point-of-care immunoassay, insulin-like growth factor binding protein-1, IGFBP-1, placental protein 12, PP12, alpha-fetoprotein, AFP

Introduction

Over the past several decades, point-of-care diagnostic testing has revolutionized the medical management of patients with emergent conditions in the acute care setting.[1,2] Rapid provision of results can facilitate sounder clinical decision making, improved patient adherence, and greater patient satisfaction, all of which lead to better clinical outcomes. In fact, an international survey of primary care physicians identified a strong clinical need and desire for a variety of point-of-care tests to inform more accurate medical management decisions in a more timely fashion.[3]

There has been a concerted effort to develop and commercialize rapid, point-of-care immunoassay tests for rupture of fetal membranes that accurately detect proteins found in high concentrations in amniotic fluid but at extremely low background concentrations in cervicovaginal secretions.[4] The first generation of these tests employed a monoclonal antibody approach focusing on insulin-like growth factor binding protein-1 (IGFBP-1, also known as placental protein 12) and placental alpha microglobulin-1.[5–10] Enthusiasm about this point-of-care approach and to more accurately diagnose rupture of membranes has led to the recent development of a combined monoclonal/polyclonal antibody immunoassay to detect two different proteins found in amniotic fluid at high concentrations.[11] The description, indications for use, procedural steps, and laboratory and clinical characterization of this assay are presented herein.

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Device description

The ROM Plus® (ROM Plus, Clinical Innovations, Salt Lake City, UT, USA) is a rapid, point-of-care, qualitative immunochromatographic test (Figure 1). This diagnostic device uses a unique monoclonal/polyclonal antibody approach to detect two different proteins found in amniotic fluid at high concentrations. ROM Plus detects alpha-fetoprotein (AFP) and IGFBP-1. The combination of IGFBP-1 and AFP was chosen not only because of its robust historical literature support as ideal protein markers for amniotic fluid but also the unique characteristics of each protein. IGFBP-1 is synthesized by the decidua of the placenta and reaches a very high concentration level in the amniotic fluid early in the first trimester and remains at that level until delivery.[12–19] However, AFP, synthesized by the fetal liver and yolk sac, reaches its peak concentration late in the second/early third trimesters.[20–25] This increases the chance that the proteins will be detected, especially in the preterm patient, when an accurate diagnosis of ruptured fetal membranes is most crucial.

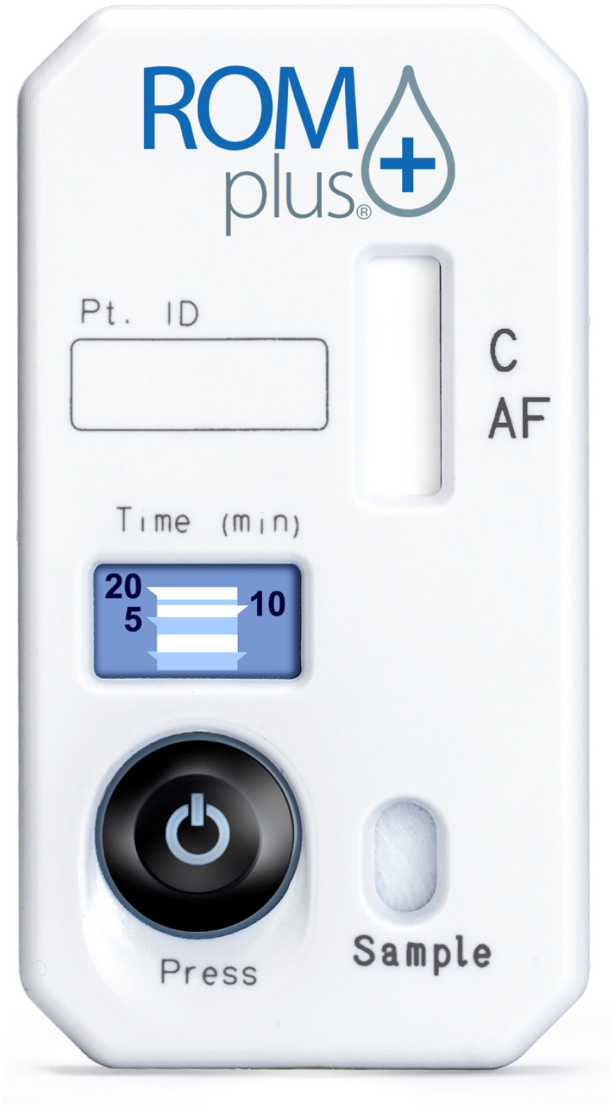

Figure 1 ROM Plus® fetal membrane rupture test.
Abbreviations: C, control; AF, amniotic fluid.

In addition to using a unique monoclonal/polyclonal antibody approach, ROM Plus provides several features designed to improve the ease of use. Unlike the first generation point-of-care immunoassays, the test strip is housed in a convenient cassette that is placed flat on the bench top reducing the risk of inadvertent sample spills. It also contains a built-in, dye-infused timer that is activated with a finger. The control samples are housed in a glass ampoule within a plastic vial with a dropper top; they do not require freezing or special handling. To activate the control, one simply breaks the glass ampoule within the vial, which releases the lyophilized protein and allows it to mix with the buffer solution. The plastic vial with dropper top is then used to dispense the sample into the well of the ROM Plus cassette.

Indications for use

The 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 rupture of membranes. The test detects AFP and IGFBP-1 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 in pregnant women in conjunction with other signs and symptoms.

Procedural details

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

Figure 2 illustrates the procedural details of the ROM Plus when used as a point-of-care test. Briefly, a fluid sample is collected by placing a swab 5–7 cm into the vagina 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 toward the absorbent pad.

During migration, the sample reacts with the mono/polyclonal antibodies on the test strip membrane. These

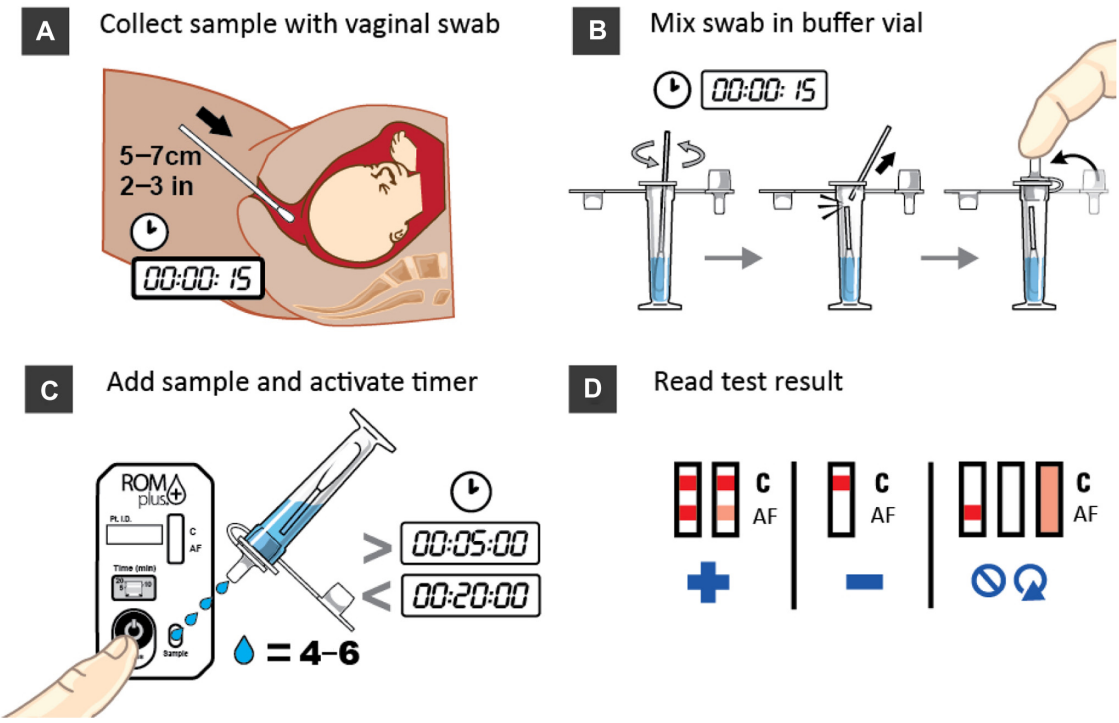

Figure 2 ROM Plus® point-of-care procedural steps.
Notes: (**A**) The sterile swab is removed from its package to collect a sample from the surface of the vagina being careful not to touch anything prior to its insertion. The swab is inserted into the vagina 5–7 cm deep and then withdrawn after a minimum of 15 seconds. (**B**) The swab tip is placed in the vial and mixed with the buffer solution. After breaking off the swab tip at the scored mark, the tip is left in the vial. For point-of-care applications, the drop dispenser lid is employed and the tip is allowed to remain in the buffer solution for a minimum of 15 seconds. (**C**) 4–6 drops of the sample/buffer solution are added to the sample well of the ROM Plus cassette and the timer is started. The results can be visualized in 5–20 minutes. (**D**) If the control line is visible (C), the test result is negative. If both the control (C) and the test line (AF) are visible, the 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.
Abbreviation: AF, amniotic fluid.

antibodies are immunoreactive to the proteins, IGFBP-1 and AFP, which are markers of amniotic fluid. As the membrane absorbs the liquid sample, a control line will appear, indicating an adequate sample was applied and the device is functioning properly.

If the sample contains IGFBP-1 and/or AFP, it binds to the antibody of the test line, causing it to appear and indicating a positive test result. If the sample does not contain IGFBP-1 and/or AFP, only the control line will be visible, indicating a negative result.

Preclinical laboratory development

The ROM Plus assay has been validated for the parameters of linearity, limit of detection, accuracy/reproducibility, sensitivity, specificity, and cross-reactivity. The "high dose hook" effect was determined to estimate ROM Plus's upper detection range. Concentrations of IGFBP-1 were tested up to 400,000 ng/mL and AFP up to 200,000 ng/mL with positive visual results for 100% of ROM Plus tests sampled. The lowest limit of detection is 5 ng/mL for IGFBP-1 and 150 ng/mL for AFP.

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 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 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 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 and shown to be negative. The IGFBP-1 assay does not cross-react with IGFBP-2, -3, or -4 on Western blot results. Finally, ROM Plus has been shown to be negative when tested with specimens that were positive for bacterial vaginosis and common sexually transmitted diseases. All samples were tested at a pH >4.5.

An external, independent evaluation of the ROM Plus was conducted at Thomas Jefferson Medical Center to investigate the analytical and operational characteristics of the assay. The sensitivity for detection and stability of controls, dilution factor of swab samples, and titer of near-term amniotic fluid and biological fluids commonly found in the vagina other than amniotic fluid were examined. The ROM Plus demonstrated excellent analytical performance and user-friendly features. Specifically, the mass-carrying capacity of the swab for a 7 g/dL albumin solution was, on average, 79±13 µL, given a diluent volume of 380 µL, indicating an average minimum dilution for samples of 18%. The positive control (stated concentrations; AFP =600 ng/mL, IGFBP-1 =20 ng/mL) was positive to 1:30 dilution, consistent with ROM Plus stated analytical sensitivity (AFP =150 ng/mL, IGFBP-1=5 ng/mL) after accounting for dilution. Also, the control (at a 1:8 titer) remained positive after 10 days of storage, either refrigerated or frozen. Amniotic fluid collected from near-term patients was positive to a titer of 1:3,000, while urine from near-term pregnant patients was negative.

Clinical diagnostic performance characteristics

Thomasino et al[11] conducted a multicenter, prospective observational study to compare the accuracy of the ROM Plus with that of current conventional clinical assessment for the diagnosis of ruptured fetal membranes. Standard clinical assessment included a speculum examination for amniotic fluid pooling, ferning, and environmental pH change using nitrazine. Rupture of membranes was diagnosed if fluid was seen leaking from the cervical os, or if two of the three conditions were present: pooling of fluid, positive nitrazine test, or ferning. Membrane rupture was confirmed on review of medical records following delivery. In 285 patients (15–42 weeks gestation), the false positive rate for the ROM Plus was 9%, false negative rate 0.5%, sensitivity 99%, and specificity 91%, with positive and negative predictive values of 85% and 99%, respectively. In comparison, the sensitivity of conventional clinical evaluation was 85%, specificity 98%, with positive and negative predictive values of 99% and 77%. Ferning's sensitivity was 99%, specificity 72%, with positive and negative predictive values of 80% and 99%. Finally, nitrazine testing had a sensitivity of 93%, specificity of 83%, and positive and negative predictive values of 90% and 88%.

Rogers et al[26] at a single clinical center, compared the diagnostic performance characteristics between two methods used for the detection of rupture of fetal membranes as measured in the same patient. Vaginal secretions were evaluated using the conventional fern test as well as the ROM Plus in 75 pregnant patients who presented with complaints of rupture of membranes. Both tests were compared to an analytical confirmation of ruptured membranes using three external laboratory tests. Diagnostic performance characteristics uniformly favored ROM Plus compared to the fern test: sensitivity (100% vs 77.8%), specificity (94.8% vs 79.3%), positive predictive value (75% vs 36.8%), negative predictive value (100% vs 95.8%), and accuracy (95.5% vs 79.1%).

Discussion

Spontaneous rupture of membranes can occur at any gestational age and presents a particularly serious clinical problem if it occurs prior to 37 weeks gestation where it is responsible for 20%–40% of preterm births.[27–29] Thus, accurate and timely diagnosis of membrane rupture is imperative to inform and guide gestational age-specific interventions to optimize perinatal outcomes and reduce the risk of serious complications, including preterm delivery and infections such as chorioamnionitis and neonatal sepsis.[14,30,31] An incorrect diagnosis of membrane rupture (ie, false positive test) can also have serious clinical ramifications, such as the initiation of unnecessary obstetrical interventions that may include hospitalization, administration of medications, and even iatrogenic premature delivery.[32]

When rupture of membranes is suspected, the diagnosis is conventionally made using the sterile speculum examination to identify leakage or pooling of amniotic fluid, coupled with microscopic evaluation of the collected specimen for evidence of ferning/crystallization and pH testing of the fluid with nitrazine test paper.[31,33,34] While this approach has remained the standard of care for decades, the results can be equivocal, especially when more than an hour has elapsed since ROM.[35] Additionally, the sterile speculum exam is both subjective and labor intensive and has been shown to have inadequate diagnostic performance characteristics for the accurate detection of ruptured membranes.[31,36–39]

The high level of diagnostic accuracy achieved with the ROM Plus is particularly important in cases of equivocal membrane rupture, as nearly one-quarter of all patients ultimately diagnosed with ruptured membranes do not present with overt clinical evidence of ruptured membranes on initial presentation.[34]

The high sensitivity consistently achieved with the ROM Plus test in clinical studies is due, in large part, to the unique monoclonal/polyclonal antibody approach where the polyclonal antibodies combine with multiple (8–12) amino acid

peptides contained in the 259 full-length IGFBP-1 protein chain, while the monoclonal tests combine with a single epitope site. This may provide an advantage over other currently available rapid immunoassay tests that rely on a single monoclonal antibody. Future comparative assessments of different immunoassays will be necessary to elucidate any diagnostic and/or procedural advantages across various commercially-available tests.

In conclusion, this unique monoclonal/polyclonal immunoassay can be performed easily and rapidly at the patients' bedside by a variety of caregivers without the need for a speculum examination.

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Author contributions

All authors contributed to the conception, execution, and drafting of the manuscript, and provided critical revision of the manuscript for intellectual content. All authors read and provided final approval of the version to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

Disclosure

The authors report no conflicts of interest in this work.

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