Tests for the Evaluation of Preterm Labor and Premature Rupture of Membranes

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Coverage Policy

Each of the following tests for the evaluation of preterm labor (PTL) is considered experimental, investigational or unproven:

- salivary estriol testing
- bacterial vaginosis (BV) testing

Each of the following tests for the evaluation of premature rupture of membranes is considered experimental, investigational or unproven:

- placental alpha-microglobulin-1 (PAMG-1) (e.g., PartoSure™, AmniSure® ROM)
- placental protein 12 (PP12)/ insulin-like growth factor binding protein (IGFBP-1) combined with alpha-fetoprotein (e.g., ROM Plus®)
- insulin-like growth factor binding protein IGFBP-1 (e.g., Actim® PROM)

Each of the following for the evaluation of pregnant women at high risk for preterm delivery is considered experimental, investigational or unproven:
- inflammatory biomarker testing, including but not limited to cytokines (e.g., interleukin-6, interleukin-8), maternal matrix metalloproteinase-9, and C-reactive protein
- hormone-related biomarker testing including but not limited to human chorionic gonadotropin and phosphorylated insulin-like growth factor binding protein-1

**Overview**

This Coverage Policy addresses testing for the evaluation of preterm labor (PTL), premature rupture of membrane (PROM), and risk of preterm delivery (PTD).

**General Background**

Preterm delivery (PTD) is defined as the birth of an infant at less than 37 weeks of gestation. The major risks of PTD to the infant are death, respiratory distress syndrome (RDS), hypothermia, hypoglycemia, necrotizing enterocolitis, jaundice, infection, and retinopathy of prematurity. Preterm labor (PTL) is defined as regular contractions associated with cervical change before the completion of 37 weeks of gestation. It is the major cause of PTD. The ability to predict whether a woman is at risk of PTD is valuable, as it allows the opportunity to administer maternal corticosteroid therapy, which decreases infant morbidity and mortality. Detecting PTL also allows for the use of maternal tocolytic therapy, which may prolong pregnancy for up to 48 hours in some women, during which time corticosteroids can be administered. Because these therapies may also have unwanted maternal and fetal side effects, the use of these therapies should be limited to women with true PTL at high risk for spontaneous preterm birth (PTB).

Maternal medical history associated with high risk of preterm labor includes a history of a previous preterm birth and a cervical length of less than 25mm. Behavioral factors include low pre-pregnancy weight, smoking, substance abuse, and short interpregnancy interval. These can be assessed and addressed with preconception care. Existing medical conditions in the pregnant woman may also increase the risk of PTL such as vaginal bleeding, urinary tract infections, genital tract infections, and periodontal disease (American College of Obstetricians and Gynecologists [ACOG], 2012). The diagnosis of preterm labor generally is based on a clinical assessment of regular uterine contractions accompanied by a change in cervical dilation, effacement, or both, or initial presentation with regular contractions and cervical dilation of at least 2 cm (ACOG, 2016).

**Preterm Labor Evaluation**

**Salivary Estriol:** Estriol levels have been shown to increase significantly 2–4 weeks before the onset of spontaneous labor. Estriol assessment has historically been accomplished through serial blood or 24-hour urine collections, the latter devised to allow for correction of diurnal hormone variations. Salivary estriol testing was developed because of the cumbersome nature of these tests. The FDA issued a PMA for SalEst™ (Adeza Biomedical Corporation, Sunnyvale, CA) in 1998. Salivary estriol has been identified as a predictor primarily of late PTB. Late PTB has low rates of neonatal morbidity and mortality and thus the test is rarely used in clinical practice (Ramsey and Andrews, 2003).

**Salivary Estriol Literature Review:** The available evidence investigating the use of salivary estriol includes a randomized controlled trial (RCT) (n=601) by Heine et al. (1999) that compared the accuracy of salivary estriol testing to that of the Creasy score for predicting PTL followed by PTB. Serial salivary estriol testing was found to correctly predict the appropriate outcome more often than the Creasy score, 91% versus 75%, respectively. Salivary estriol testing had a sensitivity of 44%, specificity of 92%, positive predictive value (PPV) of 19%, and an NPV of 98%, using two consecutive positive tests as criteria for prediction. Corresponding values for the Creasy system were 48% sensitivity, 75% specificity, 7% PPV, and 97% negative predictive value (NPV) (Heine, et al., 2000). While these study results suggest that salivary estriol testing may predict outcomes more accurately than the Creasy scoring system, the impact of salivary estriol testing on treatment decision making or patient outcomes has not been demonstrated. Additional studies are needed to establish the role of this testing method in the management of PTL and PTB.
**Bacterial Vaginosis (BV):** BV is characterized by an overgrowth of a mixture of anaerobic bacteria and mycoplasmas that replace the normal vaginal lactobacilli. BV is a common disorder, occurring in up to 20% of women during pregnancy. Most of these cases will be asymptomatic. BV may resolve spontaneously, although women with BV in early pregnancy are likely to have persistent infection later in pregnancy. BV is associated with an increased risk for spontaneous PTD (Leitich, et al., 2003). Therefore, BV testing is recommended for women who are symptomatic for infection and will benefit from appropriate antibiotic treatment. However, there is insufficient evidence to support the use of screening asymptomatic women for BV as a means of preventing PTD.

**Bacterial Vaginosis Literature Review:** Studies in the published peer-reviewed medical literature evaluating the use of BV screening for women who are asymptomatic for PTL have yielded conflicting results. A Cochrane review (n=4429) by Swadpanich et al. (2008) assessed the effectiveness and complications of antenatal lower genital tract infection screening and treatment programs in reducing PTB and subsequent morbidity. Only one study by Kiss et al. (2004) met the inclusion criteria of evaluating methods of antenatal lower genital tract infection screening compared with no screening. The primary outcome measure was PTD at less than 37 weeks. The intervention group (n=2058) had significantly lower rates of PTB than the control group (n=2097) (p=0.0001). The reviewers found evidence that infection screening and treatment programs in pregnant women may reduce PTB and preterm low birthweight. It was noted that future studies should include evaluation of gestational ages at screening tests and the effects of different types of infection screening programs (Swadpanich, et al., 2008). A Cochrane update performed by Sangkomkamhang et al. (2015) identified no additional studies for review and arrived at similar conclusions.

A Cochrane review by McDonald et al. (2005) indicates that there was no difference in the risk of PTD for any treatment versus no treatment or placebo. However, there is some evidence that treatment of BV in women with a history of PTD reduces the occurrence of preterm rupture of membranes and low birthweight. Based on these outcomes, the authors suggest that there may be some benefit in screening for BV and treatment with oral antibiotics in women who have experienced a previous PTB. However, the evidence does not demonstrate that the use of antibiotics for BV reduces PTB. There is also no evidence for outcomes for the neonate that includes survival, severe health effects and/or long-term hospitalization. A 2007 update of this Cochrane review again found little evidence that screening and treating all pregnant women with asymptomatic BV will prevent PTB and its consequences (McDonald, et al., 2007).

Okun et al. (2005) conducted a meta-analysis of RCTs (n=14 studies/6728 subjects) to determine if antibiotic therapy reduced the risk of PTB or associated adverse outcomes among pregnant women with BV or trichomonas vaginalis. Studies were included if they were RCTs in which antibiotics were compared to no antibiotic or placebo, for asymptomatic and symptomatic women with these two infections. Study participants included women who were not in labor, had intact membranes, and were in the second or third trimester of pregnancy at the time of randomization. The primary outcome measure was PTB before 37 weeks of gestation. Secondary outcome measures included PTB before 35 weeks, 34 weeks, 32 weeks, or 28 weeks of gestation, low birth weight, very low birth weight, and preterm PROM (PPROM). Studies were excluded if more than 20% of women in either group in the trial were lost to follow-up. The results of this systematic review and meta-analysis indicate that while treatment reduced the risk of persistent infection with BV or trichomonas vaginalis, the incidence of PTL was not reduced; in women with trichomonas vaginalis treated with metronidazole, the incidence of PTB was increased.

A multicenter RCT (n=4429) by Kiss et al. (2004), screened asymptomatic women for BV during a routine evaluation early in the second trimester. Pregnant women presenting for routine prenatal visits between 15 weeks plus 0 days and 19 weeks plus 6 days of gestation were enrolled in this study. Women were included if they had no subjective complaints (e.g., contractions, vaginal bleeding). Those with those with multiple pregnancies were excluded. The caregivers for the intervention group (n=2058) received the results of the testing and instituted treatment. The caregivers for the control group (n=2097) were not given any test results. The primary outcome measure was PTB at less than 37 weeks. Secondary outcome measures included PTD of < 37 weeks combined with different birth weight categories ≤ 2500 grams, and the rate of late miscarriage. The intervention group had significantly lower rates of PTB than the control group (p=0.0001). The number of preterm infants with a birth weight ≤ 2500 grams was significantly lower in the intervention group than in the control group (p=0.0002). It is possible the results were influenced by physician knowledge of patients with a risk factor for
PTL, leading to a different level of care for those with a positive result. It is unclear if factors specific to the country in which the study was conducted preclude the application of these study results to patients in the United States.

There is insufficient evidence to support the use of screening asymptomatic women for BV as a means of preventing PTD.

**Premature Rupture of Membranes (PROM) Evaluation**

Premature rupture of membranes (PROM) is rupture of membranes occurring prior to the onset of labor. Preterm PROM (PPROM) is defined as a membrane rupture that occurs before 37 weeks of gestation. Intra-amniotic infection has been shown to be commonly associated with PPROM, especially if the rupture occurs at earlier gestational ages. Risk factors for PROM include previous PTB (especially if the cause was PROM), short cervical length (less than 25 mm) during the second trimester, and PTL or symptomatic contractions in the current pregnancy. PROM can also occur without any identifiable risk factor.

Most cases of PROM can be diagnosed based on the patient's history and physical examination. Sterile speculum examination allows for visual inspection of fluid and provides an opportunity to assess for cervicitis and umbilical cord or fetal prolapse, cervical dilation and effacement, and to obtain cultures as appropriate. Digital cervical examinations add little additional information to the speculum examination and are avoided due to the increase risk of infection. The diagnosis of membrane rupture typically is confirmed by the visualization of amniotic fluid passing from the cervical canal and pooling in the vagina and a basic pH test of vaginal fluid or arborization (ferning). The pH of vaginal secretions is generally 4.5-6.0, while amniotic fluid usually has a pH of 7.1-7.3. False-positive results may occur in the presence of blood or semen, alkaline antiseptics, bacterial vaginosis, prolonged ROM and minimal residual fluid. In unusual cases additional tests may aid in the diagnosis. Ultrasonographic examination of the amniotic fluid may be useful, but is not diagnostic. Fetal fibronectin is a sensitive but nonspecific test for ruptured membranes; a negative test result is strongly suggestive of intact membranes, but a positive test result is not diagnostic of PROM. Several commercially available tests for amniotic proteins are currently on the market, with high reported sensitivity for PROM. However, false-positive test result rates of 19–30% have been reported in patients with clinically intact membranes and symptoms of labor. When the clinical history or physical examination is unclear, membrane rupture can be diagnosed unequivocally with ultrasonographically-guided transabdominal instillation of indigo carmine dye, followed by observation for passage of blue fluid from the vagina (ACOG, 2018). Other laboratory tests such as placental α-microglobulin-1 (PAMG-1) or phosphorylated insulin-like growth factor binding protein 1 (pIGFBP-1) are potential markers of an increased risk of preterm birth. However, the utility of these tests has not been validated in either large or randomized clinical trials (Lockwood, 2017).

At term, PROM complicates approximately 8% of pregnancies and is generally followed by the onset of spontaneous labor and delivery. The most significant maternal risk of term PROM is intrauterine infection. Preterm PROM complicates approximately 3% of pregnancies in the United States and is associated with 12% of all births and can result in significant neonatal morbidity and mortality (ACOG, 2018). An accurate diagnosis of PROM facilitates optimal clinical assessment and expectant management. As such, several proteins found in cervicovaginal fluid, have been proposed for the detection of PROM.

**Placental alpha-1 microglobulin**: Placental alpha-1 microglobulin (PAMG-1) is being investigated as a marker for the detection of PROM. PAMG-1 is found in high levels in amniotic fluid and low levels in cervicovaginal discharge when fetal membranes are intact.

**U.S. Food and Drug Administration (FDA)**: On April 11, 2018 the PartoSure test was granted premarket approval (PMA) as an aid to rapidly assess the risk of spontaneous preterm delivery in ≤ 7 days from the time of cervicovaginal sample collection in pregnant women with symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (<3 cm). The cervicovaginal sample should be taken between 24 weeks and 34 weeks, 6 days gestation in women with a singleton gestation.

The AmniSure ROM (rupture of fetal membrane) test was granted 510(k) approval by the FDA because it is considered to be substantially equivalent to another device already on the market. Under the FDA 510(k) approval process, the manufacturer is not required to supply to the FDA evidence of the effectiveness of the
AmniSure prior to marketing. The 510(k) summary stated that the AmniSure is substantially equivalent to the AmnioTest™. According to the FDA, the AmniSure ROM test is a rapid, non-instrumented, qualitative immunochromatographic test for the in vitro detection of amniotic fluid in vaginal secretion of pregnant women. AmniSure detects PAMG-1 protein marker of the amniotic fluid in vaginal secretions. The test is for use by healthcare professionals to aid in the detection of ROM when patients report signs, symptoms or complaints suggestive of ROM.

**PAMG-1 Literature Review:** Studies evaluating the safety and effectiveness of PAMG-1 testing to detect PROM includes cohort, observational, and uncontrolled comparative trials. In general studies are limited by non-randomized, uncontrolled design, and small patient population.

In a Hayes Directory Report on the AmniSure ROM Test for Detection of Fetal Membrane Rupture, 17 studies evaluated the AmniSure test to detect the clinical validity and one study evaluated the clinical management of the test result. Sample sizes ranged from 100–251 and included pregnant women at 11–42 weeks gestation. The accuracy of the AmniSure test was evaluated by comparing the usual combined methods (e.g., visual observation, nitrazine test, and microscopic observation), individual methods (i.e., nitrazine test), against other immunoassays (e.g., Actim PROM) and in noncomparative studies for the diagnosis of PROM. Although some of these studies found that the AmniSure test is somewhat better than the usual combined methods for diagnosis of PROM, the available studies did not provide consistent evidence that the AmniSure test is more accurate than the usual methods of testing. In addition, the available studies did not demonstrate that the AmniSure test is more accurate than other available immunoassays for diagnosis of PROM. Only one study evaluated the clinical utility and found that the use of the test provided a statistically significant increase in clinician confidence in diagnosing PROM (p<0.0001). The Directory Report noted overall the body of evidence was low quality. The main reasons for the overall low-quality rating pertaining to the clinical validity of the AmniSure test largely reflects individual study limitations and concerns regarding generalizability to clinical practice in the United States. The majority of studies regarding clinical validity (13 of 17 studies) were conducted outside of the United States in countries from Africa, the Middle East, Asia, or Central/South America. For clinical utility, the overall rating of very low is due to the paucity of evidence on the impact of this test on treatment decision making and health outcomes of women with PROM (Hayes, 2018).

Lotfi et al. (2017) conducted a prospective observational study to compare the effectiveness of a PAMG-1 test (PartoSure) and standard clinical assessment in the prediction of preterm births. Women (n=148) with singleton pregnancies between 24 0/7 and 36 6/7 weeks of gestation presenting with self-reported symptoms of preterm labor, including uterine contractions, back pain, intermittent lower abdominal pain, pelvic pressure, vaginal bleeding, and cramping were included in this study. Patients had a vaginal swab inserted without a speculum to collect the sample for PartoSure testing. Then the physician conducted a standard clinical assessment which included evaluation of the patient's history, observed symptoms, contractions measured by Cardiotocography (CTG) and a vaginal examination. The results of the standard clinical assessment determined whether patients were admitted to the hospital or discharged to home. Standard clinical assessment and the PAMG-1 test were conducted on all 148 patients. For delivery within seven days, the PAMG-1 test demonstrated the following performance metrics: sensitivity of 66.7%, specificity of 98.6%, positive predictive value (PPV) of 75.0%, and negative predictive value (NPV) of 97.9%. For delivery within 14 days, PAMG-1 demonstrated a sensitivity of 53.8%, specificity of 99.3%, PPV of 87.5%, and NPV of 95.7%. The PAMG-1 test was statistically superior to standard clinical assessment with respect to specificity for delivery within seven days (p<0.0001) and for delivery within 14 days (p<0.0001). An author-noted limitation of this study was a small number of spontaneous deliveries within seven days.

Wing et al. (2017) conducted a prospective observational study to compare the rapid bedside test for placental α-microglobulin-1 (PAMG-1) with the fetal fibronectin (fFN) test for the prediction of imminent spontaneous preterm delivery among women with symptoms of preterm labor. The study included pregnant women with symptoms suggestive of preterm labor between 24 and 35 weeks of gestation with intact membranes and cervical dilatation ≤3 cm. Of the 796 women included in the study cohort, 711 (89.3%) had both PAMG-1 and fFN results and valid delivery outcomes available for analysis. The healthcare providers were blinded to the PAMG-1 results. The primary analysis was to demonstrate that the PPV in the PAMG-1 cohort was greater than the observed PPV rate of the fFN cohort. The overall rate of preterm birth was 2.4% within seven days of testing and 4.2% within 14 days of testing with respective rates of spontaneous preterm birth of 1.3% and 2.9% respectively. Fetal
fibronectin was detected in 15.5% and PAMG-1 was detected in 2.4%. The PPVs for spontaneous preterm delivery within seven days or less among singleton gestations (n=13) for PAMG-1 and fFn were 23.1% and 4.3%, respectively (p<0.025 for superiority). The NPVs were 99.5% and 99.6% for PAMG-1 and fFN, respectively (p<0.001 for noninferiority). The limitations of the study were the rate of spontaneous preterm delivery at seven days or less and 14 days or less, 1.3% and 2.8%, respectively. After enrollment, 15% of patients were excluded for various reasons. One third (n=10) of the 30 deliveries, which occurred within 14 days of study enrollment, were excluded because they were deemed not to have been spontaneous. A second author-noted limitation was that, too few patients were enrolled with multiple gestations (n=66) or transvaginal ultrasonography (n=125) to conduct subgroup analyses.

Ng et al. (2013) conducted a prospective study (n=211) comparing the diagnostic accuracy of placental alpha microglobulin-1 assay and standard diagnostic methods for detecting rupture of membrane. At initial presentation, 187 patients (88.6%) had ruptured membranes, while 24 patients (11.4%) had intact membranes. All participants were assessed using the PAMG-1rapid immunoassay test, nitrazine test and ferning test at the initial speculum examination. PAMG-1 immunoassay confirmed rupture of membranes at initial presentation with a sensitivity of 95.7% (179 of 187), specificity of 100% (24 of 24), positive predictive value of 100% (179 of 179), and negative predictive value of 75.0% (24 of 32). The conventional diagnostic methods had a sensitivity of 78.1% (146 of 187), specificity of 100% (24 of 24), positive predictive value of 100% (146 of 146), and negative predictive value of 36.9% (24 of 65) in diagnosing rupture of membrane. Although study results suggest that PAMG-1 testing demonstrates improved accuracy, the study is limited by small sample size and lack of randomization.

A report issued by the Canadian Agency for Drugs and Technologies in Health (CADTH) examined the comparative accuracy of the AmniSure test versus the fern test for the assessment of rupture of the fetal membrane. Prospective observational studies (n=4 studies/559 subjects) designed to determine the diagnostic accuracy of AmniSure compared with conventional clinical criteria for assessing fetal membrane rupture were included in the assessment. All included studies reported the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the diagnostic tests, with reported ranges of 97%-99%, 69%-100%, 90%-100% and 90%-100%, respectively. Only one study compared AmniSure to the fern test alone. This study exclusively included term pregnancies limiting generalizability to PROM. Other studies made a comparison to a group of clinical criteria which varied between studies. The CADTH concluded that AmniSure was found to have high sensitivity and predictive accuracy for rupture of fetal membranes, however the lack of direct comparison to individual tests and limited statistical reporting prevent drawing conclusions about comparative effectiveness (CADTH, 2012).

Abdelazim and Makhlouf (2012) conducted a prospective comparative study (n=150) to evaluate the accuracy of the placental alpha microglobulin-1 (PAMG-1) (AmniSure test) in the diagnosis of premature rupture of the fetal membranes (PROM). Pregnant women after 37 weeks gestation were divided into two groups according to presence (n=75/group 1) or absence (n=75/group 2) of PROM. Women with multiple pregnancies, <37 weeks gestation, fetal distress, vaginal bleeding, preterm labor, or chorioamnionitis were excluded. All subjects received nitrazine, ferning and PAMG-1 testing. The sensitivity and the specificity of PAMG-1 to diagnose PROM were 97.33 and 98.67%, respectively, compared to 84% sensitivity and 78.67% specificity for ferning test and 86.67% sensitivity and 93.3% specificity for nitrazine test. The PPV and negative predictive value (NPV) of PAMG-1 were 98.64 and 97.37%, respectively, compared to 79.74% PPV and 83.1% NPV for ferning test and 82.28% PPV and 85.91% NPV for nitrazine test. PAMG-1 was more accurate (98%) for detection of PROM than Ferning (81.33%) or Nitrazine (84.0%) tests (Abdelazim and Makhlouf, 2012).

Phupong and Sonthirathi (2012) conducted a prospective observational study (n=100) of patients with signs or symptoms of PROM with term (n=76) and preterm (n=24) pregnancies. Conventional methods (e.g., nitrazine test, ferning test) were used to establish the diagnosis and were compared to PAMG-1 immunoassay results. The diagnosis of ROM was confirmed by reviewing the medical records after delivery. PAMG-1 immunoassay had a sensitivity of 97.2%, specificity of 69%, positive predictive value (PPV) of 90.8%, negative predictive value (NPV) of 90.9% and an accuracy of 89%. The PAMG-1 immunoassay was found to be significantly more sensitive in diagnosing ROM (97.2% versus 88.7%, p=0.031). However, the conventional combined standard methods had more specificity than PAMG-1 immunoassay (96.6% versus 69%, p=0.008). On final diagnosis, PAMG-1 immunoassay gave a false positive result in nine cases (31%), and a false negative result in two cases.
A prospective cohort study (n=199) by Birkenmaier et al. (2011) evaluated the performance of the PAMG-1 immunoassay (AmniSure) in cervicovaginal secretions of patients with uncertain ROM. Evaluation of patients included clinical assessment, examination for cervical leakage, Nitrazine test and measurement of the amniotic fluid index by ultrasound and AmniSure. ROM occurrence was based on review of the medical records after delivery. AmniSure had a sensitivity of 94.4%; specificity of 98.6%; positive predictive value (PPV) 96.2%; negative predictive value (NPV) 98.0%. Clinical assessment showed a sensitivity of 72.2%; specificity of 97.8%; PPV of 92.9%; NPV of 90.6%. AmniSure testing was reported to be more sensitive for diagnosing ROM (p=0.00596) compared to clinical assessment, independent of the examiners experience.

Tagore et al. (2010) compared insulin-like growth factor binding protein-1 (IGFBP-1), PAMG-1 and nitrazine testing to diagnose PROM. PAMG-1 was performed in 100 women with a sensitivity of 92.7%, specificity of 100%, PPV of 100% and NPV of 95.2%. IGFBP-1 was performed in 94 women with a sensitivity of 87.5%, specificity of 94.4%, PPV of 92.1% and NPV of 91.1%. In 98 women in whom nitrazine test was performed, the sensitivity was 85%, specificity was 39.7%, PPV was 49.3% and NPV was 79.3%.

A prospective observational study (n=189) Lee et al. 2007 compared the accuracy of an immunoassay to measure levels of PAM-1 in cervicovaginal secretions with that of conventional clinical assessment for the diagnosis of ROM. PAMG-1 immunoassay was found to confirm ROM initial presentation with a sensitivity of 98.7%, specificity of 87.5%, PPV of 98.1%, and NPV of 91.3%. PAMG-1 immunoassay was reported better than both the conventional clinical assessment and the nitrazine test alone in confirming the diagnosis of rupture of membranes.

Cousins et al. (2005) conducted a comparative study (n=203) of AmniSure versus standard diagnostic methods for detection of ROM in women suspected of ROM. The AmniSure test was found to have a sensitivity of 98.9%, specificity of 100%, and NPV of 99.1% in diagnosing ROM (Cousins et al, 2005). Test performance was assessed by comparing AmniSure results to clinical history, nitrazine and fern results, presence of pooling, ultrasound evidence of oligohydramnios, and findings from repeated examinations.

Study results suggest that PAMG-1 testing with AmniSure is accurate when compared to standard testing methods for PROM. However, study populations have included a wide range of gestational ages and clinical presentations. Clinical utility has not been established as no published studies have compared health outcomes in cases where treatment decisions were based on AmniSure testing versus standard testing methods.

**Alpha-fetoprotein (AFP) Combined with Placental Protein 12 (PP12)/insulin-like Growth Factor Binding Protein (IGFBP-1):** AFP is a substance made in the liver of the fetus. AFP is found in high concentrations in amniotic fluid, while being found in extremely low levels of maternal blood and cervicovaginal secretions of women with intact membranes. Insulin-like growth factor binding protein is secreted from the placenta. It is the major insulin-like growth factor binding protein in the amniotic fluid that gradually increases in the second trimester and remains higher throughout pregnancy in comparison to its plasma levels. Detection of IGFBP-1 in the cervical–vaginal secretions has been proposed as a diagnostic method for ruptured amniotic membrane (Akercan, et al., 2004). Determining levels of both AFP and PP12/IGFBP-1 in vaginal secretion is thought to be indicative of rupture of membrane.

**U.S. Food and Drug Administration (FDA):** On November 23, 2011, the ROM Plus® Fetal Membrane Rupture Test (Clinical Innovations, LLC, Murray, UT) obtained clearance from the FDA through the 510(k) approval process, as substantially equivalent to the predicate device, the AmniSure ROM test. According to the FDA, 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 ROM. The test detects AFP and PP12 or insulin growth factor binding protein from amniotic fluid in vaginal secretion. The test is to be used by health care professionals to aid in the detection of ROM in conjunction with other signs and symptoms (FDA, 2011).

**AFP and PP12/IGFBP-1 Literature Review:** There is a paucity of studies in the published peer-reviewed medical literature assessing the performance of AFP and PP12/IGFBP-1 testing. As such, there is insufficient evidence from which to draw conclusions regarding accuracy and clinical utility.
Rogers et al. (2016) reported their results of a prospective comparative study between two methods used for the detection of ROM at a single institution: (1) the fern test and (2) a monoclonal/polyclonal immunoassay test (ROM Plus®; Clinical Innovations). Patients (n=75) were pregnant between 14 and 41 weeks gestation presenting with a complaint of ROM. Clinicians performed a standard sterile speculum examination upon the patient’s presentation and a slide was sent for clinical laboratory evaluation of crystallization (fern test). A second swab was then collected from the vagina for evaluation using the ROM Plus® immunoassay test. The clinicians and patients were blinded to the results of the ROM Plus® test. Clinical decision making was based on the results of the fern test, physical examination, and the clinical course. Diagnostic performance favored ROM detection using the immunoassay test compared to the fern test: sensitivity (100% vs. 77.8%), specificity (94.8% vs. 79.3%), PPV (75% vs. 36.8%), NPV (100% vs. 95.8%), and accuracy (95.5% vs. 79.1%). Fifteen cases had discordant results between the two test measurements. Limitations of the study include comparison against only one comparator and the choice of the confirmatory test. The gold standard to confirm ROM is to inject indigo carmine into the amniotic sac during amniocentesis and then assess whether any blue fluid is visibly leaking from the cervical or pooling in the vaginal vault, this confirmation was not performed.

**Insulin-like growth factor binding protein (IGFBP–1):** IGFBP-1 alone has also been evaluated for the identification of ROM.

**U.S. Food and Drug Administration (FDA):** In 2007, the Actim PROM test (Alere™ Inc., Waltham, MA) obtained 510(k) clearance from the FDA as substantially equivalent to the AmniSure ROM test. The FDA stated that the Actim PROM test is a visually interpreted, qualitative immunochromatographic rapid test for the detection of amniotic fluid in cervicovaginal secretions during pregnancy. Actim PROM test detects IGFBP-1, which is a major protein in amniotic fluid and a marker of the presence of amniotic fluid in a cervicovaginal sample. The test is intended for professional use to help diagnose the ROM in pregnant women at >34 weeks gestation when patients report signs, symptoms or complaints suggestive of ROM or if such signs are otherwise observed. On January 9, 2014, the Actim PROM test received 510(k) clearance from the FDA for use in pregnant women ≥ weeks gestational age and for the use of vaginal swab samples collected without the use of a speculum in addition to the current sample type, swabs collected with the use of a speculum (FDA, 2014).

**IGFBP-1 Literature Review:** Studies in the published peer reviewed medical literature evaluating the efficacy of IGFBP-1 for the detection of rupture of membrane primarily consists of case series with patient populations ranging from 54-150 (Abdelazim, 2014; Bogavac, et al., 2010; Akercan, et al., 2005). These studies included pregnant women between 20-36 weeks gestation (Abdelazim, 2014; Bogavac, et al., 2010) and > 37 weeks gestation (Abdelazim, 2014), with and without confirmed PROM. Sensitivity and specificity for the test were 89.3%-100% and 82.7%-95%, respectively, with an 84% positive predictive value and 100% negative predictive value reported by Akercan et al. (2005). Limitations include study design and small sample sizes.

Tripathi et al. 2016 conducted a prospective observational study (n=468) to compare the accuracy of rapid bedside tests for phIGFBP-1 and fetal fibronectin (fFN) to predict preterm delivery among women with threatened PTL. Women with a singleton pregnancy of 28-36 weeks, intact membranes, and symptoms suggestive of PTL were included. Outcome measures of diagnostic accuracy were sensitivity, specificity, PPV, NPV, and likelihood ratio. Overall, 196 (41.9%) patients delivered preterm (< 37 weeks). For delivery before 37 weeks, the phIGFBP-1 test yielded a sensitivity, specificity, PPV, and NPV of 81.1%, 97.1%, 95.2% and 87.7%, respectively. The sensitivity, specificity, PPV, and NPV for the fFN test were 19.4%, 99.4%, 97.4%, and 63.2%, respectively. The phIGFBP-1 test demonstrated higher sensitivity and NPV than the fFN test for delivery before 34 weeks and within seven days of testing (p<0.05 overall). The likelihood ratios analysis indicated that a patient with a positive phIGFBP-1 test result was 8.5 times more likely to deliver prior to 34 weeks than a patient with a negative test result. Conversely, a patient with a negative phIGFBP-1 test result was 14 times less likely to deliver before 34 weeks than a patient with a positive test result. The positive and negative likelihood ratios for fFN were 20.50 and 1.27, respectively, for delivery < 34 weeks. Acknowledged study limitations include the very low rate of positive results for the rapid fFN test and the observational study design (Tripathi, et al., 2016). Study results suggest that the rapid bedside test for phIGFBP-1 was more reliable in the prediction of preterm delivery than fFN. However, further well-designed prospective studies with large patient populations are needed to confirm the accuracy and clinical utility IGFBP-1 testing.
Conde-Agudelo and Romero (2016) performed a systematic review and meta-analysis of the evidence (n=43 studies) evaluating cervical phosphorylated IGFBP-1 (phIGFBP-1) for the prediction of PTB. The evidence included cohort or cross-sectional studies, 15 of which provided data on asymptomatic women (n=6583 subjects) and 34 studies on women with an episode of PTL (n=3620 subjects). Case-control studies were excluded. Additionally, studies were excluded that assessed cervical phIGFBP-1 in women with suspected or established PPROM; assessed phIGFBP-1 only in vaginal secretions, amniotic fluid, or blood; reported data for cervical phIGFBP-1 only as mean or median values; or did not publish accuracy test estimates and sufficient information to calculate them could not be retrieved. For asymptomatic women, the predictive accuracy of the cervical phIGFBP-1 test for PTB at <37, <34, and <32 weeks of gestation was reported to be minimal, with pooled sensitivities and specificities ranging from 14%–47% and 76%–93% respectively. In women with an episode of PTL, the test was found to have low predictive performance for delivery within seven and 14 days of testing, and PTB at <34 and <37 weeks of gestation with pooled sensitivities and specificities that ranged from 60%–68%, 77%–81%, respectively. It was concluded that In conclusion, there is insufficient evidence to recommend the routine clinical use of the cervical phIGFBP-1 test in women with or without symptoms of PTL. Acknowledged limitations of the meta-analysis included the lack of a standard definition of PTB across studies, risk of bias in some studies, and statistical heterogeneity (Conde-Agudelo and Romero, 2016).

Although the available studies in the published peer-reviewed medical literature suggests that the accuracy of immunoassay testing of cervicovaginal placental proteins (e.g., placental alpha-microglobulin-1 [PAMG-1]); placental protein 12 [PP12]/insulin-like growth factor binding protein [IGFBP-1]) for the detection of premature rupture of membranes may be equivalent to current standard testing methods, controlled clinical trials are needed to demonstrate improved clinical utility over these methods and the impact on health outcomes.

Preterm Delivery Prediction

Inflammatory and Hormone-Related Biomarkers: It is suggested in the medical literature that intrauterine infection and inflammation play a role in spontaneous preterm deliveries. Elevated concentrations of inflammatory biomarkers such as interleukin-6 (IL-6), C-reactive protein (CRP), and matrix metalloproteinase-9 (MMP-9) have been associated with an increased risk for PTB and/or newborn morbidity. Hormone-related biomarkers (e.g., human chorionic gonadotropin and phosphorylated insulin-like growth factor binding protein-1) are also being investigated as predictors of preterm delivery. Simple, rapid, noninvasive, and safe tests of markers of asymptomatic intrauterine infection that are associated with adverse neonatal outcomes could be useful in development of strategies for risk stratification and prediction of morbidity among women with or without symptoms of labor (Sorokin, et al., 2010).

Literature Review: Studies evaluating the safety, effectiveness, and clinical utility of these biomarkers have been conducted and include observational studies and systematic reviews.

Moghaddam et al. (2012) conducted a cohort study (n=778) to examine the relationship between maternal serum CRP levels in the first 20 weeks of pregnancy and the risk of preterm PROM and PTB. Maternal serum CRP levels were measured in all subjects during the first half of pregnancy with follow-up of patients up to time of delivery. Preterm PROM and PTB were defined as the occurrence of membranes rupture and birth, respectively before 37 weeks of gestation. Of the 778 pregnant women, 19 (2.41%) developed premature PROM, and 57 (7.3%) had PTBs. CRP levels >4 mg/L had statistically significant relationships with preterm PROM and PTB. With a cut-off level of 4 mg/L of CRP, sensitivity and specificity for PTB were 81% and 70%, respectively, and for preterm PROM they were 79%, and 67%, respectively. It was noted that the role of inflammatory markers like CRP in preterm PROM and PTB is controversial and that further studies are needed to establish a definitive association.

Conde-Agudelo et al. (2011) performed a systematic review of observational studies (n=72 studies/89786 women) to evaluate the accuracy of novel biomarkers to predict spontaneous PTB in women with singleton pregnancies and no symptoms of preterm labor. For serum levels of biomarkers including interleukins-2, -6 and -10, and C-reactive protein, the pooled sensitivities and specificities ranged from 3%-49% and 51%-97% respectively. Positive and negative likelihood ratios predicting PTB before 32, 34, and 37 weeks of gestation were between 0.4 and 4.5 (median, 1.1), and between 0.6 and 1.3 (median, 1.0), respectively. For cervicovaginal levels of interleukins-6 and -8, the pooled sensitivities, specificities, varied from 24%-44% and 75%-93% (median, 83%), with positive and negative LRs from 1.1 to 4.0, and 0.6 to 1.0 respectively. For amniotic fluid
levels of biomarkers including interleukin-6, MMP-8, and C-reactive protein, the pooled sensitivities, specificities, and positive and negative LRs ranged from 12%–86%, from 43%–99%, from 0.9–40.0, and from 0.2–1.1, respectively. In summary, moderate predictive accuracy was found for 4/30 biomarkers (IL-6 and angiogenin, in amniotic fluid; human chorionic gonadotropin and phosphorylated insulin-like growth factor binding protein-1, in cervicovaginal fluid). The remaining biomarkers had low predictive accuracy. None of the biomarkers evaluated in this review met the criteria to be considered a clinically useful test to predict spontaneous PTB (Conde-Agudelo, et al., 2011).

Sorokin et al. (2010) conducted an observational study (n=475) to determine if the maternal serum concentration of IL-6, CRP, and MMP-9 in asymptomatic women at risk for PTB, was associated with an increased risk for PTB and/or neonatal morbidity. Maternal serum samples collected from patients enrolled in a multicenter randomized controlled trial of single versus weekly corticosteroids. Concentrations of IL-6, CRP, and MMP-9 were subsequently determined using enzyme-linked immunoassays. Maternal serum concentrations of IL-6 and CRP, but not MMP-9, above the 90th percentile at the time of randomization were associated with PTB less than 32 weeks.

Wei et al. (2010) conducted a systematic review of observational studies (n=17 studies/6270 participants) that reported the association between inflammatory cytokines and spontaneous PTB as an outcome in asymptomatic women. Spontaneous PTB was reported to be strongly associated with increased levels of IL-6 in mid-trimester cervicovaginal fluid (OR 3.05, 95% CI 2.00-4.67) and amniotic fluid (OR 4.52, 95% CI 2.67-7.65), but there was no association in plasma specimen (OR 1.5, 95% CI 0.7-3.0). Spontaneous PTB was also found to be strongly associated with increased CRP levels in midtrimester amniotic fluid (OR 7.85, 95% CI 3.88-15.87), but the association was weak in plasma specimen (OR 1.53, 95% CI 1.22-1.90). There were insufficient data for a meta-analysis of other inflammatory cytokines.

Although available study results are promising, there is currently insufficient evidence to support the use of inflammatory and hormone-related biomarkers as predictors of PTB in women with intact membranes who are not in labor.

**Professional Societies/Organizations**

**American College of Obstetricians and Gynecologists (ACOG):** The ACOG guideline on Prelabor Rupture of Membranes states that the optimal approach to clinical assessment and treatment of women with term and preterm PROM remains controversial. According to ACOG, most cases of PROM can be diagnosed on the basis of the patient’s history and physical examination. The guideline further states that several tests for amniotic proteins are currently available with high reported sensitivity for PROM. However, these tests should be considered ancillary to standard diagnostic methods due to reported false-positive rates of 19%–30% in patients with clinically intact membranes and symptoms of labor (ACOG, 2018).

The ACOG practice bulletin on the prediction and prevention of PTB states that specific tests such as fetal fibronectin screening and bacterial vaginosis testing have been proposed to assess a woman’s risk of preterm delivery. “However, available interventional studies based on the use of these tests for screening asymptomatic women have not demonstrated improved perinatal outcomes. Thus, these methods are not recommended as screening strategies (ACOG, 2012).

**U.S. Preventive Services Task Force (USPSTF):** The USPSTF guideline on screening for BV in pregnancy concluded that the evidence is insufficient to recommend for or against routinely screening high-risk pregnant women for BV. The USPSTF recommended against routinely screening average-risk asymptomatic pregnant women for BV. It was stated that study results were conflicting and that although the magnitude of benefit exceeded risk in several studies, the single largest study evaluated reported no benefit among high-risk pregnant women (USPSTF, 2008). In a subsequent update, the USPSTF restated that pregnant women at low risk for PTD should not be screened for BV and maintained that the current evidence is insufficient to assess the balance of benefits and harms of screening for BV in pregnant women at high risk for PTD (USPSTF, 2014).

**Use Outside of the US**

Guidelines on screening and management of bacterial vaginosis in pregnancy have been prepared by the Infectious Diseases Committee of Society of Obstetricians and Gynaecologists of Canada (SOGC). The SOGC
recommended testing and the treatment of bacterial vaginosis with oral or vaginal antibiotics in symptomatic pregnant women. Asymptomatic women without risk factors for preterm birth should not have routine screening and treatment of BV. It is recommended that women at increased risk for preterm birth may benefit from routine screening for and treatment of bacterial vaginosis. In addition, testing should be repeated one month after treatment to ensure that cure was achieved (Yudin et al., 2017).

The National Institute for Clinical Excellence (NICE) (United Kingdom) guideline for the management of preterm labor and birth stated that in woman reporting symptoms suggestive of P-PROM, offer a speculum examination to look for pooling of amniotic fluid. If pooling is observed, do not perform any diagnostic test but offer care consistent with the woman having P-PROM. If pooling of amniotic fluid is not observed, consider performing an insulin-like growth factor binding protein-1 test or placental alpha-microglobulin-1 test. If the results are positive, don’t use the test results to decide what care to offer the woman. NICE advised to take into account her clinical condition, her medical and pregnancy history and gestational age, and offer care consistent with the woman having P-PROM. They recommended not to use nitrazine to diagnose P-PROM (NICE, 2015).

**Coding/Billing Information**

**Note:**
1. This list of codes may not be all-inclusive.
2. Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement.

### Salivary Estriol Testing and Bacterial Vaginosis (BV) Testing

**Considered Experimental/Investigational/Unproven:**

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<tr>
<td>82677</td>
<td>Estriol</td>
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<tr>
<td>87480</td>
<td>Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique</td>
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<tr>
<td>87510</td>
<td>Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, direct probe technique</td>
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<tr>
<td>87512</td>
<td>Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, quantification</td>
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<tr>
<td>87660</td>
<td>Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique</td>
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<tr>
<td>87800</td>
<td>Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique</td>
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<td>S3652</td>
<td>Saliva test, hormone level; to assess preterm labor risk</td>
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**Premature Rupture of Membrane Testing (e.g., AmniSure® ROM, ROM Plus®, Actim® PROM)**

**Considered Experimental/Investigational/Unproven:**

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<tr>
<td>84112</td>
<td>Evaluation of cervicovaginal fluid for specific amniotic fluid protein(s) (eg, placental alpha microglobulin-1 [PAMG-1], placental protein 12 [PP12], alpha-fetoprotein), qualitative, each specimen.</td>
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**Biomarker Testing**
Considered Experimental/Investigational/Unproven:

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<td>83516</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method</td>
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<tr>
<td>83518</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, single step method (eg, reagent strip)</td>
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<td>83519</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, by radioimmunoassay (eg, RIA)</td>
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<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified</td>
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<tr>
<td>84702</td>
<td>Gonadotropin, chorionic (hCG); quantitative</td>
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<td>84703</td>
<td>Gonadotropin, chorionic (hCG); qualitative</td>
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<tr>
<td>86140</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>87799</td>
<td>Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism</td>
</tr>
</tbody>
</table>


References


9. Canadian Agency for Drugs and Technologies in Health (CADTH). Rapid Response Report: Summary with Critical Appraisal. AmniSure versus Fern Testing to Assess the Rupture of Fetal Membranes in


