



# Medical Coverage Policy

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

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### Related Coverage Resources

- [Recurrent Pregnancy Loss: Diagnosis and Treatment](#)
- [Ultrasound in Pregnancy \(including 3D, 4D and 5D Ultrasound\)](#)

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## Overview

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

## Coverage Policy

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

- salivary estriol testing
- bacterial vaginosis (BV) testing in asymptomatic women

**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., 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 tests for predicting the risk of preterm birth in pregnant women is considered experimental, investigational or unproven:**

- placental alpha-microglobulin-1 (PAMG-1) (e.g., PartoSure™)
- phosphorylated insulin-like growth factor-binding protein-1 (phIGFBP-1) (e.g., Actim® Partus)
- serum-based proteomic testing (e.g., PreTRM®)

**Each of the following for the evaluation of pregnant women at high risk for preterm birth 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

## General Background

Preterm delivery (PTD) is defined as the birth of an infant at less than 37 weeks of pregnancy. The major risks of PTD to the infant are death, respiratory distress, 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 pregnancy. 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 chance to administer maternal corticosteroid therapy, which decreases infant illness and death. 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 given. Because these treatments may also have unwanted maternal and fetal side effects, the use of these treatments 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

pregnancy spacing 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.

PTB rates are higher for non-Hispanic Black and Indigenous women than for White, Asian, or Hispanic women in the United States. Additionally, the odds of PTB were lowest in couples in which both parents were White and steadily increased with Black parentage. Non-Hispanic Black women who have a higher level of education experience higher preterm birth rates when compared to non-Hispanic White, Asian, or Hispanic women with a lower level of education. A possible explanation for the preterm birth disparity between African-American or Afro-Caribbean women and women of other racial and ethnic backgrounds is chronic stress related to long-term exposure to structural racism. Premature infants have increased risks of sickness and death throughout childhood, particularly during the first year of life. The risks increase as the fetal age at birth decreases (Robinson & Norwitz, 2023; American College of Obstetricians and Gynecologists [ACOG], 2021).

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; Reaffirmed 2020).

### **Preterm Labor Screening**

**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).

**Literature Review - Salivary Estriol:** 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.

**Literature Review - Bacterial Vaginosis:** There is insufficient evidence that screening and treating asymptomatic BV during pregnancy prevents PTB. Meta-analyses of randomized trials performed in the general obstetric populations have generally found that treatment of asymptomatic infection does not reduce the incidence of preterm labor or delivery in the overall obstetrical population (Klebanoff, et al., 2023; Sobel, 2022).

Lee et al. (2019) conducted an unblinded, cluster-randomized controlled trial in two subdistricts of Sylhet, Bangladesh. The study assessed the effect of a community-based antenatal screening and treatment program for abnormal vaginal flora and urinary tract infections in early pregnancy (13–19 weeks) on population-level rates of preterm birth. Eligible participants within clusters were married females aged 15–49 years who became pregnant during the study period. Clusters were randomly assigned (1:1) to the intervention (n=4840) or control (n=4872) group. In both groups, community health workers made home visits to identify pregnant females and provide antenatal and postnatal care. Between 13–19 weeks' gestation, participants in the intervention group received home-based screening for abnormal vaginal flora and urinary tract infections. A random 10% (n=481) of the control group also received the intervention to examine the similarity of infection prevalence between groups. If present, abnormal vaginal flora (i.e., Nugent score  $\geq 4$ ) was treated with oral clindamycin and urinary tract infections with cefixime or oral nitrofurantoin). Both infections were retreated if persistent. The primary outcome measured was preterm livebirths before 37 weeks' gestation. Secondary outcomes measured all pregnancy outcomes before 37 weeks' gestation, low birthweight, small for gestational age, maternal clinical urinary tract infection after 20 weeks' gestation, and neonatal mortality. The primary analysis included 3818 livebirths in the intervention group and 3557 livebirths in the control group. In the intervention group, the prevalence of abnormal vaginal flora was 16.3% and that of urinary tract infection was 8.6%. The effective coverage of successful treatment in the intervention group was 58% in participants with abnormal vaginal flora, and 71% in those with urinary tract infections. Overall, the incidence of preterm livebirths before 37 weeks' gestation did not differ significantly between the intervention and control groups (21.8% vs 20.6%). Author noted limitations included: testing for infections was done before 20 weeks' gestation, only two infections were targeted, the study findings might not be generalizable to other regions and the method of establishing gestational age could be considered a limitation (menstrual calendar). The authors concluded that the treatment of asymptomatic bacteriuria and urinary tract infections had no effect on preterm birth or low birthweight.

Bellad et al. (2018) performed a randomized double blind placebo controlled trial to evaluate whether oral clindamycin reduces the risk of preterm birth (PTB) in women with abnormal vaginal microflora as evidenced by a vaginal pH  $\geq 5.0$ . Women were required to have a singleton fetus between 13 0/7 weeks and 20 6/7 weeks gestation with an elevated vaginal pH ( $\geq 5.0$ ) by colorimetric assessment. The patients (n=1727) were randomized into two groups. The clindamycin group (n=866) received oral clindamycin 300 mg twice daily for five days and the placebo group (n=861) received an identical-appearing placebo. The primary outcome measured was the incidence of delivery before 37 weeks gestation. Follow-up occurred 7–28 days following administration of the medication along with a standardized assessment of symptoms. Data on background characteristics and outcomes were then serially collected using a standardized registry at birth and 42 days post-completion. The rate of PTB before 37 weeks was comparable between the two groups (clindamycin 13.9% versus placebo 13.8%, between-group difference 0.2% [p=0.93]) as was PTB at less than 34 weeks (clindamycin 4.8% versus placebo group 4.6%, between-group difference 0.3% [p=0.81]). There were no differences in the incidence of birthweight of < 2500 g, < 1500 g, miscarriage, stillbirth or neonatal death. Author acknowledged limitations included using vaginal pH as a marker for an abnormal vaginal microbiome to target low resource settings and not performing a screening ultrasound. The study concluded that treating BV with oral clindamycin did not decrease PTB among women with vaginal pH  $\geq 5.0$ .

Subtil et al. (2018) published the results of a double blind randomized controlled trial (PREMEVA) which evaluated whether treatment of bacterial vaginosis decreased late miscarriages or spontaneous very preterm birth. Women with a low risk pregnancy aged 18 years or older with bacterial vaginosis and a gestational age less than 15 weeks were included in the study. Patients (n=2869) were randomly assigned to three parallel groups: single-course of clindamycin (n=943), triple-course of 300 mg clindamycin twice-daily for four days (n=968), or placebo (n=958). The primary outcome was a combination of late miscarriage (16–21 weeks) or spontaneous very preterm birth (22–32 weeks), which was assessed in all patients with delivery data (modified intention to treat). The secondary outcomes measured included hospitalization for threatened preterm delivery or premature rupture of membranes (PROM), number of hospitalization days for preterm PROM or PROM, prenatal signs of chorioamnionitis, abruptio placentae, PROM (< 37 weeks and ≥ 37 weeks), preterm delivery at 22–36 (+ six days), hyperthermia during labor, maternal post-partum fever, abscess of abdominal wall or episiotomy (post-partum), and the need for antibiotic treatment more than 24 hours after delivery. Participant data was collected at inclusion and after delivery. The incidence of the primary outcome in the patients with delivery data was not clinically significant between groups (p=0.82). None of the secondary outcomes among mothers and among newborn babies differed significantly between the two groups. The groups had similar rates of spontaneous preterm delivery between 22 and 36 weeks' gestation (4.8% in the clindamycin group and 4.1% in the placebo group) and total preterm delivery (6.7% in the clindamycin group and 5.9% in the placebo group). There were not any serious adverse events reported, however adverse events were more common in the clindamycin groups than in the placebo group (3.0% vs 1.3% respectively). Author noted limitations included compliance, the absence of test-of-cure study design and the low prevalence of very preterm delivery in our population. The study demonstrated that systematic screening and subsequent treatment for bacterial vaginosis in women with low-risk pregnancies showed no evidence of risk reduction of late miscarriage or spontaneous very preterm birth.

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. A Cochrane update performed by Sangkomkarn et al. (2015) identified no additional studies for review and arrived at similar conclusions.

A Cochrane review by McDonald et al. (2003) assessed the use of antibiotics for treating BV in pregnancy. The study indicated 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 2013 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 (Brocklehurst, et al., 2013).

Additional evidence evaluating if the treatment of BV with antibiotic therapy reduced the risk of PTB among pregnant women is primarily in the form of RCT's, systematic reviews and meta-analysis with patient populations ranging from 4429–10,513. The studies included asymptomatic and symptomatic women with BV that compared antibiotic therapy to no antibiotic therapy or placebo. The primary outcome of the studies was PTD before 37 weeks of gestation. In general, the results of these trials suggested that treating bacterial vaginosis with oral metronidazole or vaginal clindamycin before 28 weeks of pregnancy did not reduce the incidence of preterm labor (Rebouças et al., 2019; Okun et al., 2005; Kiss, et al., 2004).

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

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

Premature rupture of membranes (PROM) is rupture of membranes occurring prior to the onset of labor. Preterm PROM (PPROM) is defined 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, 2020).

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 2–3% of pregnancies in the United States and is associated with 10% of all births and can result in significant neonatal morbidity and mortality (ACOG, 2020). 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-microglobulin-1:** Placental alpha-microglobulin-1 (PAMG-1) is being investigated as a marker for the detection of PROM. PAMG-1 is found in high levels in amniotic fluid.

**U.S. Food and Drug Administration (FDA):** 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 health care professionals to aid in the detection of ROM when patients report signs, symptoms or complaints suggestive of ROM.

**Literature Review - PAMG-1:** Studies evaluating the safety and effectiveness of PAMG-1 testing for the detection of PROM includes cohort, observational, systematic reviews with meta-analysis, retrospective reviews and uncontrolled comparative trials. In general studies are limited by non-randomized, uncontrolled design, and small patient population.

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).

Additional evidence evaluating the diagnostic accuracy of PAMG-1 (AmniSure) is primarily in the form of prospective studies with patient populations ranging from 150–211. Studies compared PAMG-1 (AmniSure) to clinical assessment, nitrazine test, ferning and measurement of the amniotic fluid index by ultrasound. They reported the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PAMG-1 (AmniSure). With reported ranges of 94.4%–98.7%, 87.5%–100%, 96.2%–100%, 75.0%–98%, respectively (Esin, et al., 2021; Ng, et al., 2013; Abdelazim and Makhlof, 2012; Birkenmaier, et al., 2011; Lee, et al., 2007). Study results suggested 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, also called placental protein 12 (PP12), 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 (Akerca, et al., 2005). 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 PPI12 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).

**Literature Review - AFP and PP12/IGFBP-1:** 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.

Sean Esplin et al. (2020) conducted a prospective, observational evaluation on the efficacy of immunoassays in diagnosing spontaneous rupture of membranes (SROM). Included subjects (n=324) had a singleton pregnancy ≥ 15 weeks with suspected SROM. The primary outcome was a final diagnosis of SROM at 48 hours after initial evaluation. Two immunoassays designed to diagnose SROM (ROM Plus® and AmniSure®) were compared to standard clinical assessment (sterile speculum exam (SSE) for nitrazine ferning and pooling) and ultrasound as needed. For the identification of SROM at 48 hours after presentation among all study subjects, ROM Plus had a sensitivity of 91.7%, specificity of 97.0%, a PPV of 94.8% and an NPV of 95.1%, whereas AmniSure had a sensitivity of 93.4%, a specificity of 95.0%, a PPV of 91.9% and a NPV of 96.0%. Clinical exam at time of presentation in these women had a similar sensitivity (87.5%) specificity (100%), PPV (100%) and NPV (98.3%) compared to the performance of the immunoassays. Performance of the two commercially available immunoassays was statistically equivalent. There was also no significant difference in sensitivities and specificities between the ROM Plus, AmniSure and standard clinical assessment.

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).

**Literature Review - IGFBP-1:** 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.

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%.

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.

### **Predicting the Risk of Preterm Birth**

The strongest predictors of preterm birth is a prior preterm birth, prior PPROM, multiple gestation, and a short cervix. The assessment of cervical length in the second trimester has been shown to identify women at increased risk for preterm birth. Cervical shortening early in the second trimester is more predictive of preterm delivery than shortening beyond 24 0/7 weeks of gestation (ACOG, 2021).

Laboratory tests such as placental  $\alpha$ -microglobulin-1 (PAMG-1), phosphorylated insulin-like growth factor binding protein 1 (pIGFBP-1) or serum proteins 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, 2023; Robinson and Norwitz, 2023).

**Placental alpha-microglobulin-1:** Placental alpha-microglobulin-1 (PAMG-1) is being investigated as a marker for predicting the risk of preterm delivery. Low levels of PAMG-1 is found 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  $\leq 7$  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.

**Literature Review - PAMG-1:** 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 PAMG-1 test (PartoSure) 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  $\leq 3$ 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.

Studies evaluating the safety and effectiveness of PAMG-1 (e.g., PartoSure™) testing to predict the risk of preterm birth includes cohort, observational, systematic reviews with meta-analysis and

uncontrolled comparative trials (Cnota et al., 2022; Kashanian et al., 2022; Pirjani et al., 2021). In general studies are limited by non-randomized, uncontrolled design, and small patient population. High quality randomized controlled trials comparing PAMG-1 with other well-established modalities are needed.

**Phosphorylated Insulin-Like Growth Factor-Binding Protein-1 (phIGFBP-1) (e.g., Actim® Partus):** phIGFBP-1 is being investigated as a marker for predicting the risk of preterm delivery. A positive test result indicates the presence of tissue damage, which may lead to preterm delivery. A negative test result, indicates that tissue damage is not present and delivery is unlikely within the next 1–2 weeks.

**U.S. Food and Drug Administration (FDA):** There were not any FDA approvals located for phIGFBP-1 (e.g., Actim® Partus) testing to predict the risk of preterm birth.

**Literature Review - Actim® Partus:** Melchor et al. (2018) performed a systematic review and meta-analysis of the evidence (n=65 studies) evaluating the accuracy of placental alpha microglobulin-1 (PAMG-1), fetal fibronectin (fFN) and phosphorylated insulin-like growth factor-binding protein-1 (phIGFBP-1) tests in predicting spontaneous preterm birth within seven days of testing in women with symptoms of preterm labor. The evidence included prospective or cohort studies that provided data on PAMG-1 (n=14 studies; n=2278 patients), fFN (n=40 studies; n=7431 patients) and phIGFBP-1 (n=22 studies; n=3192 patients). The inclusion criteria was patients before 37 weeks' gestation with signs or symptoms suggestive of PTL with clinically intact membranes and minimal ( $\leq 3$  cm) cervical dilatation. Studies were included if the objective was to determine the accuracy of specified biomarkers in predicting PTB and information was sufficient to calculate test performance metrics for the prediction of spontaneous PTB within seven days of testing. The Bi-variate mixed model pooled sensitivity, specificity, PPV, NPV of PAMG-1 was 76%, 97%, 76.3%, 96.6%, 22.51, respectively. The Bi-variate mixed model pooled sensitivity, specificity, PPV, NPV of fFN was 58%, 84%, 34.1%, 93.3, respectively. The Bi-variate mixed model pooled sensitivity, specificity, PPV, NPV, of phIGFBP-1 was 93%, 76%, 35.2%, 98.7%, respectively. The authors concluded that in the prediction of spontaneous PTB within seven days of testing in women with signs and symptoms of preterm labor, the PPV of PAMG-1 was significantly higher ( $p < 0.05$ ) than that of phIGFBP-1 or fFN. The other diagnostic accuracy measures did not differ between the three biomarker tests. Author acknowledged limitations noted that the study may be under-powered, as these researchers were unable to attain convergence in the low-risk and intermediate-risk groups and the three risk groups showed variation in sensitivity and specificity. Additionally, the biomarkers were not studied in the same number of subjects and only 45% of studies were considered to have a low risk of bias. Finally, this review did not examine the impact of test performance on patient management and resource economics.

Tripathi et al. 2016 conducted a prospective observational study (n=468) to compare the accuracy of rapid bedside tests 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 included the very low rate of positive results for the rapid fFN test and the observational study design (Tripathi, et al., 2016). Study results suggested 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 PPRM; 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.

**Serum-Based Proteomic Testing:** Using proteomic technology, Sera Prognostics developed a biomarker signature for predicting spontaneous preterm birth (sPTB). The PreTRM test measures maternal serum for insulin-like growth factor-binding protein 4 (IBP4) and sex hormone-binding globulin (SHBG) and is proposed to predict an individual's risk of sPTB. The serum blood test is indicated for asymptomatic patients, before symptoms of preterm labor.

**U.S. Food and Drug Administration (FDA):** There were not any FDA approvals located for PreTRM testing to predict the risk of preterm birth.

**Literature Review - Serum-Based Proteomic Testing:** Branch et al. (2021) conducted a prospective, randomized controlled trial that evaluated if mid-trimester serum proteomics screening of women at low risk for spontaneous preterm birth (sPTB) and the use of a PTB risk-reduction protocol in those whose results indicated an increased risk of sPTB would reduce the incidence of sPTB. Women, aged 18 years or older with singleton pregnancy were eligible for participation if they had a cervical length > 2.5 cm at the time of mid-trimester ultrasound, had no medical contraindications to continuing pregnancy, and had intact membranes with no signs and/or symptoms of preterm labor. The authors noted that 91.7% of participants were white (Hispanic and non-Hispanic) and less than 1% Black or African-American. Over 80% were married, 79% had commercial insurance, and over 45% had a 4-year college degree. Patients (n=1191) were randomized to a screening group using the the PreTRM test (n=595) (Sera Prognostics, Salt Lake City, UT) or the control group without screening (n=596). The control group, the screen-negative women and those with unusable blood samples received standard obstetric care in their community setting. The screen positive women (n=198) were offered the sPTB risk-reduction protocol that included two visits to a preterm birth prevention clinic (at < 24weeks and at 26–30 weeks). The risk reduction protocol was designed to include several interventions (including progesterone supplementation, cervical length surveillance, daily low dose aspirin, and weekly contact for review of symptoms). The protocol that was offered to screen-positive women included. The primary outcome measured the rate of sPTB < 37 weeks. The

secondary outcomes measured gestational age at delivery, total length of neonatal stay, and NICU length of stay (LOS). Ten patients were lost to follow-up. Spontaneous PTB < 37 weeks occurred in 2.7% of screened women and 3.5% of controls ( $p=0.41$ ). There were no between-group differences in the gestational age at delivery, total length of neonatal stay, and NICU LOS. However, the NICU LOS among infants admitted for sPTB was significantly shorter ( $p=0.005$ ) in the screened group. Author noted limitations included the early termination of the study due to inadequate funding and the rate of sPTB was just over 3%, much lower than the expected rate. Although there was a statistical difference in the NICU LOS among neonates admitted after sPTB, a difference likely due to a reduced number of infants in the screened group that delivered < 35 weeks. Lastly, the results may not be generalizable to the general U.S. population because our study population lacks ethnic diversity and over 75% of participants had commercial insurance. The study concluded that serum proteomic screening of a low risk population coupled with a sPTB risk-reduction protocol in screen positive patients did not result in a significantly lower rate of sPTB < 37 weeks. The findings of a shortened NICU LOS following sPTB in the screened group should be studied further.

Markenson et al. (2020) conducted The Multicenter Assessment of a Spontaneous Preterm Birth Risk Predictor study (TREETOP) which was a prospective observational study that assessed the performance of the ratio of insulin-like growth factor-binding protein 4 (IBP4) and sex hormone-binding globulin (SHBG) to predict spontaneous and medically indicated very preterm births, neonatal morbidity and/or mortality, and the length of hospital stay of the neonate. This is the first phase of a planned 2 phase study which aims to expand the clinical utility of the previously validated IBP4/SHBG PTB biomarker (Saade et al., 2016). The study enrolled women ( $n=5011$ ) at low risk for PTB aged 18 years and older with singleton pregnancies experiencing no symptoms of preterm labor or membrane rupture. Women were enrolled from 17 weeks, 0 days to 21 weeks, 6 days gestational age (GA). Gestational age was confirmed by a first trimester ultrasound. Eight hundred and forty-seven women were included in this substudy analysis. Maternal blood was drawn between 19 weeks, one day and 20 weeks, six days gestation, with the results of the remaining study participants ( $n=2807$ ) blinded for future validation studies. Neonatal morbidity and mortality were analyzed using neonatal morbidity index (NMI). NMI scores of 0-3 reflect increasing numbers of morbidities or length of neonatal intensive care unit stay and 4 represents perinatal mortality. Clinical data were recorded on four pre-specified occasions across pregnancy and neonatal outcomes were measured through 28 days of life. Deliveries were classified as term ( $37^{0/7}$  weeks) or preterm (<  $37^{0/7}$  weeks) with the specific gestational age at birth captured. There were nine preterm birth cases at < 32 weeks', 0 days gestation; 21 of 847 infants had NMI scores of 3, and 4 of 21 infants had an NMI score of 4. The ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio was substantially higher in both preterm births at < 32 weeks' 0 days gestation and there were more severe neonatal outcomes. The ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio was significantly predictive of birth at < 32 weeks' 0 days gestation ( $p=0.016$ ). The ratio of insulin-like growth factor-binding protein 4 to sex hormone-binding globulin ratio predicted neonatal outcomes with respective area under the receiver operating characteristic curve of 0.67 ( $p=0.005$ ) and 0.78 ( $p=0.026$ ) for NMI scores of 3 or 4. Additionally, the ratio of insulin-like growth factor-binding protein 4 to sex hormone binding globulin significantly stratified neonates with increased length of hospital stay (log rank  $p=0.023$ ). Author noted limitations included the low power for precise determination and optimization of threshold parameters, related to the number of early PTB cases and the excess of iatrogenic over spontaneous PTBs. Additionally, this study was limited in evaluating this marker for spontaneous birth at < 32 weeks' gestation. Additional limitations were that the strongest predictor performance occurred in a relatively narrow blood draw range ( $19^{1/7}$  to  $20^{6/7}$  weeks) and that the predictor used only one clinical covariate (BMI) associated with PTB risk (e.g., omitting maternal age, history of PTB, cervical length).

Saade et al. (2016) conducted the Proteomic Assessment of Preterm Risk (PAPR) prospective study to develop and validate the performance of the insulin-like growth factor-binding protein 4 (IBP4) and sex hormone-binding globulin (SHBG) ratio as a predictor for sPTD in asymptomatic pregnant women. Women (n=5501) were enrolled between 17 weeks, 0 days and 28 weeks, 6 days gestational age (GA). Maternal blood was collected at enrollment and was processed by a proteomic workflow, and proteins were quantified by multiple reaction monitoring mass spectrometry. Following delivery, data were collected for maternal and infant outcomes and complications. All deliveries were classified by the study sites as term ( $\geq 37^{0/7}$  weeks GA), spontaneous preterm delivery (including preterm premature rupture of membranes [PPROM]), or medically indicated preterm births (miPTB). Strict blinding and independent statistical analyses were used. The authors reported that the predictor had an area under the receiver operating characteristic curve (AUROC) value of 0.75 and sensitivity and specificity of 0.75 and 0.74, respectively. The IBP4/SHBG predictor at this sensitivity and specificity had an odds ratio of 5.04 for spontaneous preterm delivery. Accuracy of the IBP4/SHBG predictor increased using earlier case vs control gestational age cutoffs (e.g.,  $< 35^{0/7}$  vs  $35^{0/7}$  week's gestational age). Additionally, higher-risk subjects defined by the IBP4/SHBG predictor score generally gave birth earlier than lower-risk subjects. Author noted study limitations included: the majority of study centers did not perform a transvaginal ultrasound measurement of cervical length (CL), women with prior preterm delivery who were not being treated with progesterone was insufficient to allow inclusion of this variable in the analysis. The study concluded that a serum-based molecular predictor identifies asymptomatic pregnant women at risk of spontaneous preterm delivery. Additional studies are needed to support the outcomes of this study.

There is insufficient evidence to support the use of serum-based proteomic testing (e.g., PreTRM<sup>®</sup>) to predict the risk of preterm delivery in asymptomatic pregnant women.

Although the available studies in the published peer-reviewed medical literature suggests that the accuracy of immunoassay and serum testing to predict the risk of preterm birth 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.

### **Evaluation of Pregnant Women at High Risk for Preterm Birth**

**Inflammatory and Hormone-Related Biomarkers:** It is suggested in the medical literature that intra uterine 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 - Inflammatory and Hormone-Related Biomarkers:** Studies evaluating the safety, effectiveness, and clinical utility of these biomarkers have been conducted and include observational studies and systematic reviews.

Hornaday et al. (2022) conducted a systematic review to identify maternal blood biomarkers that may be predictive of spontaneous preterm birth (sPTB). Seventy-seven articles met the inclusion criteria (48 case-control and 29 cohort studies) reporting 278 unique markers significantly associated with and/or predictive of sPTB in at least one study. The most frequently investigated biomarkers were those measured during maternal serum screening for aneuploidy, including pregnancy associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), alpha

fetoprotein (AFP), and estriol. Low levels of PAPP-A were associated with sPTB (OR range 1.7–5.4). The evidence suggesting an association between maternal levels of hCG and sPTB was inconclusive, though some studies suggest that low levels of hCG were associated with increased risk of sPTB (OR range 0.8–2.0) and that high levels of hCG independently decreased risk of sPTB. High serum AFP was significantly associated with sPTB (OR range 1.9–8.3), although one study showed no significant difference. Studies on serum estriol were split, while two studies showed a significant association between high estriol and sPTB, others found no association. Based on the synthesized evidence, no single biomarker was clearly predictive of sPTB. The authors reported that some biomarkers were specific to ethnicity. The United States of America was the most common country of study origin, with the majority of study participants being non-Hispanic Caucasian or Black. Corticotropin releasing hormone (CRH) was associated with sPTB in a majority ethnic Chinese population and majority non-Hispanic Caucasian population, but not in a majority Black population. Serum ferritin was associated with sPTB in Indian and Egyptian populations, though results were mixed for Caucasian and Black populations in American and European countries, which may suggest socio-demographic and ethnicity specific interactions within this biomarker. Additionally, it has been suggested that cortisol is associated with sPTB in Caucasian, but not Black populations. Author acknowledged limitations included: the potential for misclassification bias due to poor outcome definitions, most studies did not provide reference to primary records that define the sPTB outcome; a number of studies failed to provide repeatable protocols on how sPTBs were differentiated from those that were medically indicated and lastly the extent of missing data may have affected the risk of bias within the reported results. The authors concluded that currently there are no known predictive biomarker for sPTB. Inflammatory and immune biomarkers show promise, but positive reporting bias limits the utility of results. Omics-style studies are promising for the identification of novel (and multiple) biomarkers. Additional well-designed studies with larger patient population are needed to identify a set of biomarkers predictive of sPTB.

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 LR 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 LR 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.

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 stated that the optimal approach to clinical assessment and treatment of women with term and preterm PROM remains challenging. According to ACOG, most cases of PROM can be diagnosed on the basis of the patient's history and physical examination. The guideline further stated 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, 2020).

The ACOG practice bulletin on the prediction and prevention of spontaneous PTB stated 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. Additionally, the ACOG stated that using serum biomarkers, genital tract microbiome, salivary hormone and protein concentrations, cervical texture, and genetic profiling for preterm birth risk assessment should be considered investigational and using in clinical practice is not recommended (ACOG, 2021).

**U.S. Preventive Services Task Force (USPSTF):** In 2020 the USPSTF updated its 2008 recommendations on screening for bacterial vaginosis in pregnancy to prevent preterm delivery. The USPSTF recommended against screening asymptomatic pregnant women who are not at risk for preterm delivery for bacterial vaginosis. The USPSTF also concluded that the current evidence is conflicting and insufficient to assess the balance of benefits and harms of screening asymptomatic pregnant women who are at increased risk for preterm delivery for bacterial vaginosis (USPSTF, 2020).

## **Use Outside of the US**

The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) practice guidelines on the role of ultrasound in the prediction of spontaneous preterm birth stated that sonographic measurement of cervical length (CL) is an essential part of screening for preterm birth (PTB). The ISUOG mentioned that if transvaginal ultrasound (TVS) CL is between 15 or 20mm and 29 mm, a laboratory test can be considered, if available. Management decisions should be based on the lab tests as follows: if result is positive, manage as for CL < 15mm or < 20 mm; if result is negative, manage as for CL ≥ 30mm. The Society considered this as a good practice point, which is when the evidence for the intervention is still lacking (Coutinho, et al., 2022).

In 2022 the Society of Obstetricians and Gynaecologists of Canada (SOGC) published updated guidelines on the diagnosis and management of preterm prelabor rupture of membranes. The guidelines stated that the diagnosis of preterm prelabor rupture of membranes (PPROM) should be based on the combination of patient's history and physical examination by a sterile speculum with direct visualization of fluid in the posterior fornix. There are multiple conventional tests (nitrazine, ferning test, and ultrasound evaluation of amniotic fluid volume) that should be considered to confirm the diagnosis of preterm prelabor rupture of membranes when amniotic fluid is not visible at speculum examination. If the diagnosis remains uncertain, ultrasound guided amniocentesis with indigo carmine dye infusion can be performed as the gold standard for the diagnosis of PPRM. Additionally, the Society stated that commercial tests, particularly placental alpha microglobulin-1 (PAMG-1), should be considered following conventional tests in equivocal cases or used as the primary tests in rural and remote areas if other diagnostic options are not available or feasible. The guideline further noted that the available evidence for commercial testing should be considered with caution because the studies were observational with small patient population and there was no comparison between the commercial tests and a reliable gold standard test (Ronconi et al., 2022).

The Society of Obstetricians and Gynaecologists of Canada (SOGC) guideline on sonographic cervical length in singleton pregnancies: techniques and clinical applications recommended that transvaginal sonography is the preferred approach for cervical assessment to identify women at increased risk of spontaneous preterm birth. The SOGC stated that it is a safe and effective technique and can be offered to women at increased risk of preterm birth (Lim, et al., 2020).

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 on biomarker testing to help diagnose preterm labor in women with intact membranes stated that routine adoption of Actim Partus and PartoSure cannot be recommended to help diagnose preterm labor in women with intact membranes when transvaginal ultrasound measurement of cervical length is not available or acceptable. There is insufficient evidence and further research is needed on the accuracy of the tests and their effect on clinical outcomes. Centers using the tests are encouraged to take part in studies to address the research considerations which can include: the impact of gestational age on the accuracy of the tests, how the tests affect clinical decision-making and the effect of the tests on outcomes for mother and baby (NICE, 2018).

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, perform 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; updated 2022).

## Medicare Coverage Determinations

	Contractor	Determination Name/Number	Revision Effective Date
NCD		No Determination found	
LCD		No Determination found	

Note: Please review the current Medicare Policy for the most up-to-date information. (NCD = National Coverage Determination; LCD = Local Coverage Determination)

## Coding Information

### Notes:

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:

CPT®* Codes	Description
<b>81513</b>	<b>Infectious disease, bacterial vaginosis, quantitative real-time amplification of RNA markers for Atopobium vaginae, Gardnerella vaginalis, and Lactobacillus species, utilizing vaginal-fluid specimens, algorithm reported as a positive or negative result for bacterial vaginosis</b>
<b>82677</b>	<b>Estriol</b>
<b>87480</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique</b>
<b>87481</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique</b>
<b>87482</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification</b>
<b>87510</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, direct probe technique</b>
<b>87511</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, amplified probe technique</b>
<b>87512</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, quantification</b>

<b>CPT®* Codes</b>	<b>Description</b>
<b>87660</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique</b>
<b>87661</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, amplified probe technique</b>
<b>87800</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique</b>
<b>87801</b>	<b>Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique</b>

<b>HCPCS Codes</b>	<b>Description</b>
<b>S3652</b>	<b>Saliva test, hormone level; to assess preterm labor risk</b>

**Considered Experimental/Investigational/Unproven when used to report premature rupture of membrane testing (e.g., AmniSure® ROM, ROM Plus®, Actim® PROM) or when testing to predict the risk of preterm delivery (e.g., PartoSure™, Actim® Partus, PreTRM®):**

<b>CPT®* Codes</b>	<b>Description</b>
<b>84112</b>	<b>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</b>
<b>0066U</b>	<b>Placental alpha-micro globulin-1 (PAMG-1), immunoassay with direct optical observation, cervico-vaginal fluid, each specimen</b>
<b>0247U</b>	<b>Obstetrics (preterm birth), insulin-like growth factor-binding protein 4 (IBP4), sex hormone-binding globulin (SHBG), quantitative measurement by LC-MS/MS, utilizing maternal serum, combined with clinical data, reported as predictive-risk stratification for spontaneous preterm birth</b>

### **Biomarker Testing**

**Considered Experimental/Investigational/Unproven:**

<b>CPT®* Codes</b>	<b>Description</b>
<b>83516</b>	<b>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method</b>
<b>83518</b>	<b>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, single step method (eg, reagent strip)</b>
<b>83519</b>	<b>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, by radioimmunoassay (eg, RIA)</b>
<b>83520</b>	<b>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified</b>
<b>84702</b>	<b>Gonadotropin, chorionic (hCG); quantitative</b>
<b>84703</b>	<b>Gonadotropin, chorionic (hCG); qualitative</b>
<b>86140</b>	<b>C-reactive protein;</b>

CPT®* Codes	Description
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism

\*Current Procedural Terminology (CPT®) ©2022 American Medical Association: Chicago, IL.

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