PRO-LAB DIAGNOSTICS

PRO·M*complete*™

(for *in vitro* diagnostic use)

PRODUCT CODE **PL.950-1** Σ 1
PL.950-5 Σ 5
PL.950-10 Σ 10

INTENDED USE

PRO·M*complete*™ is a simple and rapid qualitative immunochromatographic test for *in vitro* detection of IGFBP-1 (**I**nsulin-like **G**rowth **F**actor-**B**inding **P**rotein-1) and AFP (**A**lpha-**F**eto**P**rotein) from amniotic fluid in a vaginal swab sample.

PRO·M*complete*™ is a point-of-care test intended to aid in the detection of the rupture of fetal membranes in pregnant women reporting signs and/or symptoms of possible membrane rupture. The product is available in three sizes providing sufficient materials for one, five, or ten tests.

SUMMARY AND EXPLANATION

Premature rupture of membranes (PROM) complicates approximately 8% of pregnancies and is generally followed by the prompt onset of spontaneous labour and delivery. Preterm PROM complicates only 2% of pregnancies but is associated with 40% of preterm deliveries and can result in significant neonatal morbidity and mortality[1]. Intra-amniotic infection has been shown to be commonly associated with preterm PROM, especially at earlier gestational ages[2]. Currently, the diagnosis of PROM is based primarily on the patient's history and physical examination. The diagnosis of membrane rupture typically is confirmed by the visualization of amniotic fluid passing from the cervical canal and pooling in the vagina; a basic pH test of vaginal fluid; or arborization (ferning) of dried vaginal fluid, which is identified under microscopic evaluation[2].

PRO·M*complete*™ proposes an alternative tool to aid in the diagnosis of PROM by identification of Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1) and Alpha-FetoProtein (AFP). The addition of AFP increases the sensitivity of PRO·M*complete*™ over testing IGFBP-1 alone.

PRINCIPLE OF THE TEST

A sample of vaginal fluids is obtained with a sterile swab. After sampling the swab is placed into the extraction buffer vial, the sample liquid is expressed from the swab and the sample is then added to a lateral-flow device containing paired monoclonal antibodies specific for IGFBP-1 and AFP. One of each pair is immobilized on nitrocellulose at a marked test line. The second antibody of each pair is labelled with colloidal gold. The extracted sample migrates through the lateral flow device and the colloidal gold-labelled antibodies bind to IGFBP-1 and AFP that may be present in the sample. As the sample migrates over the immobilized monoclonal antibodies the IGFBP-1 or AFP, now labelled with colloidal gold, are bound to the immobilized line of monoclonal antibodies creating a visible line for each marker. A rabbit anti-mouse immunoglobulin is also bound to the nitrocellulose distal to the IGFBP-1 and AFP lines and captures gold-labelled monoclonal antibodies whether or not IGFBP-1 or AFP are present resulting in a visible control line (C) confirming that the test reagents are performing properly and adequate sample has been added.

REAGENTS / MATERIALS PROVIDED

Catalogue #	Description	PL.950-1 (1 test)	PL.950-5 (5 tests)	PL.950-10 (10 tests)
PL.951	Individually packaged AFP/IGFBP-1 Cassette in a foil pouch containing desiccant – each cassette contains mouse monoclonal antibodies specific for AFP and IGFBP-1 as well as rabbit polyclonal antibodies specific for mouse immunoglobulin.	1 cassette	5 cassettes	10 cassettes
PL.952	Extraction Buffer –Each vial contains 1 ml buffer with preservative	1 vial	5 vials	10 vials
520CS01	Individually packaged sterile Swabs	1 swab	5 swabs	10 swabs

MATERIALS REQUIRED BUT NOT PROVIDED

- A standard laboratory timer.

STORAGE AND STABILITY

The kit should be stored in a dry environment either refrigerated or at room temperature (2-30°C). Under these conditions the kit is stable until the indicated expiry date. Once the foil pouch containing the test cassettes is opened the test should be performed **within one hour**.

PRECAUTIONS AND WARNINGS

- "**For *in vitro* diagnostic use.**"
- Do not open the foil pouch until it reaches room temperature to avoid condensation.
- Do not use the kit beyond the expiry date.
- All patient specimens should be handled as potentially infectious. After use, dispose of the extracted patient's swab and the test cassette appropriately.
- The kit components are intended for single use.
- Do not interchange or mix reagents from different kits and lots.
- Do not use the cassette if the foil pouch is already opened or is damaged.
- The device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

SPECIMEN COLLECTION AND PREPARATION

Use the sterile swab to collect vaginal secretions. Open the swab package and place the swab into the vagina (approximately 5 cm depth) for at least 15 seconds. This process does not require a speculum.

TEST PROCEDURE

1. The test kit components and samples should be tested at room temperature.
2. Open the extraction buffer vial by twisting off the black cap and stand it vertically.
3. Dip the swab containing the vaginal secretions into the extraction buffer vial and rotate for 10 seconds. Press the swab against the vial walls in order to expel as much liquid as possible from the swab and then discard the swab. Close the tube by twisting on the black cap.
   Note: the sample can be stored for up to 6 hours at room temperature prior to proceeding with the next step.
4. Shake the extraction buffer vial to mix the contents and tap the base to ensure the liquid is in the bottom of the vial.
5. Open the foil pouch containing the test cassette and place it on a flat surface.
6. Twist off the clear dropper cap from the extraction vial, hold it vertically above the sample well (S) on the cassette, and dispense 3 drops of the extracted sample by applying gentle pressure to the walls of the vial. Avoid air bubbles in the sample well or splashes of liquid onto the results window.
7. Start the timer. As the test begins to work, you will see a reddish coloured liquid moving across the membrane.
8. Read the result after 10 minutes. Some samples may be obviously positive before 10 minutes.
   Note: do not interpret the test result after 15 minutes have elapsed.
9. Dispose of the components of the test and the sample according to procedures for potentially infectious waste.

QUALITY CONTROL PROCEDURES

- Internal procedural controls are included in the test. A coloured line appearing in the control zone (C) indicates that sufficient specimen volume has been loaded and that the operator has followed the correct procedure.
- An external positive control sample containing IGFBP-1 and AFP (sold separately) is available (PL.953).

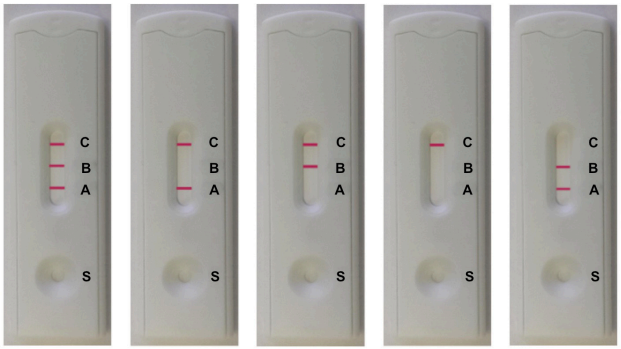
INTERPRETATIONS OF RESULTS

- The test result is considered to be positive if either the IGFBP-1 (B) or the AFP (A) band or both are present. Even weakly intense bands should be considered as a positive result. If neither of these bands are present the test result is considered to be negative.
- The control band, line C, must be present in order for the test to be valid. In the absence of a control band, review the procedure and repeat it with a new device.
- Examples of positive, negative, and invalid test results are shown on the following page. Note: line colour may vary from red to purple depending on the sample.

Canada ☎ 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit #4, Richmond Hill, ON, L4B 1K3

U.S.A. ☎ 800 522 7740 Fax 800 332 0450
21 Cypress Blvd., Suite 1070, Round Rock, Texas, 78665-1034

U.K. ☎ 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside CH62 3QL



Positive Positive Positive Negative Invalid

LIMITATIONS OF THE PROCEDURE

- Test results should be used in conjunction with clinical indications of membrane rupture.
- Presence of significant amounts of blood in the sample can lead to false positive results.
- False negative results may appear when the test is performed more than 12 hours after appearance of symptoms.
- The vaginal secretions sample swab must be placed in the extraction vial immediately after collection of the sample to prevent proteolytic breakdown of the markers being tested.

PERFORMANCE CHARACTERISTICS

Laboratory studies determined the limit of detection and reproducibility of PRO·M*complete*™. Additional studies determined test specificity and the effect of potentially interfering substances.

- The limit of detection for IGFBP-1 was 20 ng/ml and the limit of detection for AFP was 5.0 ng/ml. The assay range was 20-200,000 ng/ml for IGFBP-1 and 5 -200,000 ng/ml for AFP.
- Reproducibility was determined with clinical staff at three different trial sites. Three staff at each site tested masked samples that were negative, borderline negative, borderline positive, and positive in triplicate. All test results at the three clinical sites were consistent with expected results demonstrating that clinicians perform the test reproducibly.
- Potentially interfering substances including bodily fluids (urine from pregnant donors in the third trimester and seminal fluid both at a final concentration of 10%; blood from pregnant donors in the third trimester at a final concentration of 2%), medications (hydrocortisone, aspirin, and Tylenol at a final concentration of 0.1% , and hygiene products (body wash, shampoo, moisturizer at a final concentration of 0.1%) were evaluated for their effect on PRO·M*complete*™ performance. Of the substances tested only blood at higher concentrations had an interfering effect as it led to false positive results.
- Specificity studies showed that PRO·M*complete*™ did not react with proteins related to the target molecules (IGFBP-2, 3, 4, 5, 6 at 5 µg /ml) or known to be abundant in vaginal secretions (antitrypsin, albumin, alpha-type glycoprotein, HCG, gamma globulin, transferrin, haptoglobin, macroglobin, lactogen at 5 µg/ml).

A multi-centre prospective study evaluated the sensitivity and specificity of PRO·M*complete*™ in clinical settings. The study tested 324 pregnant women with signs and/or symptoms of premature rupture of membranes. We determined the sensitivity and specificity of PRO·M*complete*™ relative to clinical diagnosis. Discordant test results (false positive or false negative test results) were resolved by post-delivery chart review with patients who delivered within 24 hours resolved to be true positives. The study population comprised pregnant women at 15-42 weeks of gestation with signs and/or symptoms of premature rupture of membranes.

One clinician collected the vaginal secretions sample using the supplied sterile swab and placed the sample into the supplied extraction buffer vial. The sample was then transferred to another clinician, unaware of the patient diagnosis, who then performed and interpreted the test. This was done to prevent possible bias from affecting test interpretation.

The combined study results from 324 patients are shown in the table below. Initially 28 patients were positive in PRO·M*complete*™ but negative based on clinical diagnosis. Subsequent chart review indicated that 15 of those patients delivered within 24 hours and they were thus reclassified as true positive results.

PRO·M-complete™ Result	Clinical Diagnosis		PRO·M-complete™ Result	Clinical Diagnosis/ Delivery Within 24 Hours	
	Positive	Negative		Positive	Negative
Positive	192	28	Positive	207	13
Negative	7	97	Negative	7	97
Sensitivity: 96.5% (92.6-98.4%)* Specificity: 77.6% (69.1-84.4%) PPV: 87.3% (82.0-91.2%) NPV: 93.3% (86.1-97.0%)			Sensitivity: 96.7% (93.1-98.6%) Specificity: 88.2% (80.3-93.3%) PPV: 94.1% (89.9-96.7%) NPV: 93.3% (86.1-97.0%)		

*95% confidence intervals

The combined clinical study results based on clinical diagnosis/delivery within 24 hours and stratified by gestation age range are shown in the table below.

Gestational Age Range*	Sensitivity	Specificity	PPV	NPV
<34 weeks	100% (73.2-100%)** (14/14)	90.6% (73.8-97.5%) (29/32)	82.4% (55.8-95.3%)	100% (85.4-100%)
34-36 weeks	96.7% (80.9-99.8%) (29/30)	100% (83.4-100%) (25/25)	100% (85.4-100%)	96.1% (78.4-99.8%)
≥37 weeks	96.4% (92.0-98.5%) (161/167)	80% (64.9-89.9%) (36/45)	94.7% (89.9-97.4%)	85.7% (70.8-94.1%)

*gestational age information was not available for 11 patients
**95% confidence intervals

BIBLIOGRAPHY

1. **Alexander JM et al.** Clinical course of premature rupture of the membranes. Semin Perinatol. 1996; 20:369-374.
2. **Medina TM et al**. Preterm premature rupture of membranes: diagnosis and management. Am Fam. Physician 2006; 73:659-664.

= Use by

LOT = Lot number

REF = Catalogue number

= Manufacturer

= Contains sufficient for <n> tests

IVD = In vitro diagnostic medical device

= Temperature limitation

= Consult instructions for use

Revision: 2017 09