NADAL® PROM Amniotic Fluid Test

(Ref. 431006N-01/431006N-03/431006N-10/431006N-20)

1. Intended Use

The NADAL® PROM Amniotic Fluid Test is an immunochromatographic rapid test for the qualitative detection of IGFBP-1 (Insulin-like Growth Factor Binding Protein 1), a major protein marker of the amniotic fluid, in a vaginal swab. The test is intended as an aid in the diagnosis of the rupture of fetal membranes in pregnant women and designed for professional use only.

2. Introduction and Clinical Significance

Premature rupture of membranes (PROM) is relatively frequent and occurs in 5 to 10% of all pregnancies. It may lead to premature delivery and fetal infection. Conventional clinical examinations cannot always detect a leakage of amniotic fluid. In some cases, biological tests are required to confirm the suspicion of PROM. Biological tests are based on the detection of alkalinization of vaginal secretion (an easy-to-carry-out, sensitive, inexpensive procedure, but with poor specificity) or on the detection of molecules present in high concentrations in amniotic fluid (diamine oxidase, alpha feto protein, fibronectin or IGFBP-1). Detection of PROM with a simple rapid test usable at the point of care enables an immediate decision-making.

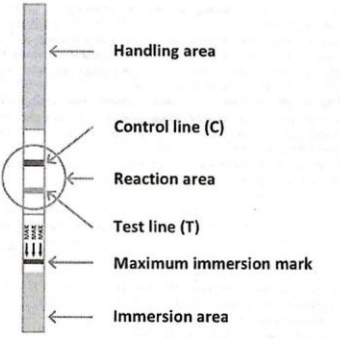
Failure to diagnose patients with PROM may result in the failure to implement salutary obstetric measures (e.g. neonatal sepsis occurs if PROM is detected more than 32 hours after initial occurance). On the other hand, a false diagnosis of PROM can lead to inappropriate interventions (e.g. hospitalization, induction of labour). Therefore, a correct and timely diagnosis of this disorder by the clinician is crucial.

However, accurate diagnosis of PROM remains a challenge for obstetrics. Unfortunately, there is no 'gold standard' for diagnosis of PROM in the clinical practice. Most of the commonly available tests are either inaccurate or to some degree invasive.

The NADAL® PROM Amniotic Fluid Test solves all of these problems. It is a rapid test intended to detect the rupture of fetal membranes, providing highly accurate and timely diagnosis of PROM. Consequently, swift measures can be taken to prevent complications (prophylactic use of antibiotics, tocolytic drugs or corticosteroids, labour induction etc.).

3. Test Principle

The NADAL® PROM Amniotic Fluid Test enables the detection of Insulin-like Growth Factor Binding Protein 1 (IGFBP-1) in vaginal secretion through visual interpretation of colour development on the test strip. Anti-IGFBP-1 antibodies are immobilised in the test line region of the membrane. During the test, the specimen reacts with further anti-IGFBP-1 antibodies conjugated to coloured particles and precoated onto the conjugate pad of the test cassette. The mixture then migrates along the membrane by capillary action and interacts with the reagents on the membrane. If there is sufficient IGFBP-1 in the specimen, a coloured line will form in the test line region of the membrane. The presence of this coloured line indicates a positive result, while its absence indicates a negative result. The appearance of a coloured line in the control line region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.



4. Reagents and Materials Supplied

Depending on the packaging unit, the NADAL® PROM Amniotic Fluid test kit contains:

- 1/3/10/20 test strips, individually packed with desiccant
- Provided additional material according to 93/42/EEC: 1/3/10/20 sterile Copan flocked swabs (FLOQSwabs™) CE 0123,

  Copan Italia S.p.A.,
  Via Perotti 10, 25125 Brescia, Italy
- 1/3/10/20 collection tubes with extraction buffer
- 1 tube holder (except Ref. 431006N-01)
- 1 package insert

5. Additional Materials Required

- Timer

6. Storage & Stability

The NADAL® PROM Amniotic Fluid Test should be stored at 2-30°C until the expiry date printed on the sealed foil pouch. The test strip must remain in the sealed foil pouch until use. Do not freeze tests. Care should be taken to protect components of the test kit from contamination. Do not use the test if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

7. Warnings and Precautions

- For professional *in-vitro* diagnostic use only.
- Carefully read through the test procedure prior to testing.
- Do not use the test beyond the expiration date indicated on the package.
- Do not use the test if the foil pouch is damaged.
- Do not reuse tests.
- The test strip should remain in the sealed foil pouch until use.
- Do not dip the test strip beyond the maximum immersion mark.
- Do not add samples to the reaction area (result area).
- In order to avoid contamination, do not touch the reaction area (result area) or the immersion area.

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- Avoid cross-contamination of specimens by using a new extraction tube for each specimen obtained.
- Do not substitute or mix components from different test kits.
- Do not swap caps between different tubes with extraction buffer.
- Do not eat, drink or smoke in the area where specimens and test kits are handled.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being assayed.
- Handle all specimens as if they contain infectious agents. Observe established precautions for microbiological risks throughout all procedures and standard guidelines for the appropriate disposal of specimens.
- The test kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled in accordance with usual safety precautions (e.g., do not ingest or inhale).
- Do not use swabs from damaged pouches.
- Humidity and temperature can adversely affect test results.
- Used testing materials should be discarded according to local regulations.

8. Specimen Collection and Preparation

Use a sterile Copan flocked swab to collect a sample of vaginal secretions from the vaginal wall. Carefully insert a swab into the vagina approximately 5 cm deep for 1 minute. Specimen collection from the cervix should be avoided.

Alternatively, a speculum can be used and vaginal secretion can be collected by placing the swab in contact with the vaginal wall at the level of the posterior fornix for 15 seconds.

9. Test Procedure

Ensure that the test components and samples are at room temperature (15-30°C) prior to testing.

1. Hold the collection tube containing extraction buffer on the lid and shake it thoroughly so that the whole liquid reaches the bottom of the tube. Open the collection tube and place it vertically on a flat, horizontal surface. Use the tube holder as an aid.

2. Dip a swab into the tube and rotate it for 10 seconds. Press the swab against the tube walls in order to squeeze as much liquid as possible from it. Discard the swab.

3. Dip a strip vertically into the tube, ensuring the arrows are pointing downwards, so that it touches the bottom of the tube. This will enhance migration of the sample. Leave the test strip in this position.

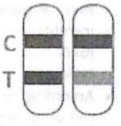
4. Remove the test strip after 5 minutes. Read the results by placing the test strip on a clean, dry and flat surface. Do not interpret test results after more than 10 minutes.

Note: For a sample collected during a strong leakage of amniotic fluid, a positive result can become visible earlier, while for a sample collected during a very small leakage, it will take the full 10 minutes for a positive result to appear.

10. Result Interpretation

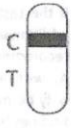
Positive

Two coloured lines appear on the membrane. One line appears in the control line region (C) and the other line appears in the test line region (T). The result indicates that the concentration of IGFBP-1 is above the detection limit of the test.



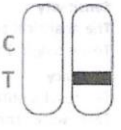
Negative

A coloured line appears in the control line region (C). No apparent coloured line appears in the test line region (T). The result indicates that the concentration of IGFBP-1 is below the detection limit of the test.



Invalid

The control line (C) fails to appear. Results from any test which has not produced a control line at the specified reading time must be discarded. Please review the procedure and repeat the test with a new test strip. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



Note:

The colour intensity in the test line region (T) may vary depending on the concentration of the analyte present in the specimen. Therefore, any shade of colour in the test line region should be considered positive. Note that this is a qualitative test only and it cannot determine the concentration of the analyte in the specimen.

Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for the control line failure.

11. Quality Control

An internal procedural control is included in the test strip:

A coloured line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Good laboratory practice (GLP) recommends the use of control materials to ensure proper test kit performance.

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12. Limitations

- The NADAL® PROM Amniotic Fluid Test is for professional *in-vitro* diagnostic use and should be only used for the qualitative detection of IGFBP-1.
- The NADAL® PROM Amniotic Fluid Test only detects the presence of IGFBP-1 in the specimen and should not be used as the sole criterium for the diagnosis of PROM.
- In pregnant women, the IGFBP-1 concentration in the blood is much lower than in the amniotic fluid, meaning that a definitive diagnosis is normally possible. However, if the sample material has been contaminated by blood, false positive results may occur which cannot be attributed to membrane rupture.
- The epithelial cells in the cervix may contain IGFBP-1 concentrations which may cause a false positive test result even without the presence of premature rupture of membranes. This also applies to non-pregnant women, as IGFBP-1 is a major protein in the endometrium around mid-cycle.
- According to existing theories (e.g. 'double sac'), ruptures can occur which may lead to a temporary loss of amniotic fluid. Tests carried out at different times can therefore deliver different results.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended.
- As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

13. Performance Characteristics

Sensitivity

The analytical sensitivity of the NADAL® PROM Amniotic Fluid Test is 5 ng/mL IGFBP-1.

Accuracy

The results obtained using the NADAL® PROM Amniotic Fluid Test were compared to those of another commercially available PROM rapid test in a multi-center clinical evaluation. The results of the study demonstrated 97.7% accuracy of the NADAL® PROM Amniotic Fluid Test when compared to the PROM rapid test of a different manufacturer.

		PROM rapid test of a different manufacturer		
		Positive	Negative	Total
NADAL® PROM Amniotic Fluid Test	Positive	83	3	86
	Negative	2	133	135
	Total	85	136	221

Sensitivity: 97.6%

Specificity: 97.8%

Overall agreement: 97.7%

Interfering substances/microorganisms

The following substances and microorganisms have been evaluated at the concentrations listed below. None of them were found to affect the test performance of the NADAL® PROM Amniotic Fluid Test.

Substance/microorganism	Concentration
Pevaryl®	**30 mg/mL**
GYNO-TROSYD®	**20 mg/mL**
Flagyl®	**100 mg/mL**
Canesten®	**40 mg/dL**
Glucose	**8 mg/mL**
Candida albicans	**11.2 x 10^8 CFU/mL**
Gardnerella vaginalis	**8.6 x 10^8 CFU/mL**
Neisseria gonorrhoeae	**10.6 x 10^8 CFU/mL**
Baby powder	**10 mg/mL**
Vagisan® Myco Kombi (Creme)	**1 mg/mL**
Baby oil	**1 mg/mL**
Medical silicone oil	**13 g/mL**

Note: blood of pregnant women may cause false positive results.

Intra-lot and inter-lot variability

Negative and positive controls at concentrations of 5 ng/mL and 25 ng/mL were tested in 10 replicates with 3 independent lots on 3 consecutive days. Each control was recognized correctly.

14. References

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