AMNIOQUICK® DUO +

Rapid test for detection of IGFBP-1 (Insulin-like Growth Factor-Binding Protein 1) and AFP (Alpha-FetoProtein).

INTENDED USE
AMNIOQUICK® DUO + is a simple and rapid immunochromatographic test that allows in vitro detection of IGFBP-1 (Insulin-like Growth Factor-Binding Protein 1) and AFP (Alpha-FetoProtein) from a vaginal swab sample. AMNIOQUICK® DUO + test is intended to detect the rupture of fetal membranes in pregnant woman with suspected rupture from a vaginal swab sample. Each test is used to obtain a qualitative result.

The AMNIOQUICK® DUO + Test Device can be used as an aid to initiate or attend therapeutic treatments by physicians. Each device is designed for professional and in-vitro diagnostic.

INTRODUCTION
The Premature Rupture of Membranes or PROM is relatively frequent and concerns 5 to 10 % of pregnancy cases. It might lead to preterm delivery and fetal infection. The leakage of amniotic liquid is not always detectable by conventional clinical examination and therefore confirmatory biological test is sometimes useful. Biological tests are based on detection of alkalisation of vaginal fluid (easy to proceed, sensitive, inexpensive but poorly specific) or presence of a molecule which is physiologically present in high concentration in amniotic fluid (diamine oxidase, alpha-fetoprotein, fibronectin, IGFBP-1).

TEST PRINCIPLE
A pair of monoclonal antibodies anti-IGFBP-1 is used for the IGFBP-1 detection. One is immobilized on the nitrocellulose membrane at the level of the B test line, it corresponds to the capture antibody. Another one is labelled with colloidal gold for the subsequence revelation.

A similar principle is used for the AFP detection with a pair of monoclonal antibodies anti-AFP, the capture antibody being immobilised at the level of the A test line, and the other one being labelled with colloidal gold for the subsequent revelation.

During the sample migration, AFP and IGFBP-1, if present in the sample, will form antigen-antibody complexes with the labelled antibodies. These complexes will be captured by the capture antibodies on the A and B test lines, creating one or two purple coloured lines generated by gold nanoparticles.

The presence of a purple internal control line in the upper part of the membrane indicates that the result is valid and that the followed procedure is correct.

MATERIALS PROVIDED
- Cassettes packed in individual pouch containing desiccant bag
- Vaginal swabs flocked with Sterile Nylon
- Dropper bottles with extraction buffer
- Instructions for use
- Patient cards

MATERIAL REQUIRED BUT NOT PROVIDED
Stop watch with alarm

STORAGE AND STABILITY
AMNIOQUICK® DUO + test is packed in aluminium pouch with desiccant. The kit should be stored in a dry area at 2-30°C. Under these conditions the test is stable until the indicated expiry date. The cassettes must be protected from humidity. Once the pouch is open, the test should be performed within 1 hour maximum.

PRECAUTIONS
- For diagnostic in-vitro use only
- For best results, strictly follow the test procedure and storage instructions.
- Do not open the foil pouch until it reaches room temperature to prevent formation of condensation. Humidity and high temperature can affect results.
- Do not use the kit beyond the expiration date.

Do not eat, smoke, or drink while handling specimens and test.
- Use white coat, disposable gloves and ocular protection while handling potentially infectious material and performing the assay.
- All patient samples should be handled as potentially infectious. When performing the test, all materials used should be treated as if they were potentially infectious. Then, eliminate the components of the test and the sample according to suitable procedure for potentially infectious waste.
- Avoid splashes and aerosol formation. Clean up spills thoroughly using an appropriate disinfectant.
- Cassettes and tubes provided in the kit are intended for single use. Do not reuse the tests, swabs, or dilution tubes.
- Do not interchange or mix reagents from different kits and lots.
- Do not use a cassette if the foil pouch is opened or damaged.
- There is no significant result variation when using 2 or 4 drops instead of 3.

SPECIMEN COLLECTION AND HANDLING
Use the sterile Nylon swab to collect secretions on the vaginal wall. Open the swab bag and place the swab into the vagina (5 cm depth) for 1 minute. Alternatively, speculum may be used and vaginal secretion may be collected by leaving the swab into contact with the vaginal wall at the level of the posterior fornix for 15 seconds.

TEST PROCEDURE
1. Bring the complete kit and samples to be tested to room temperature prior to testing.
2. Open the unit dose vial and lay it vertically on a flat and horizontal surface.
3. Dip the swab into the unit dose vial and rotate for 10 seconds. Press the tube walls in order to expel efficiently as much liquid as possible from the swab, and then discard it or break off the swab tip into the vial.
4. Close the tube with the cap and shake the collection tube. Using a piece of tissue, break the upper part of the collection tube with a twisting motion. Hold the collection tube vertically and dispense 3 drops of solution into the round sample well of the test device by applying a gentle pressure to the walls of the tube. Avoid air bubbles in the sample well or splashes of liquid into the result window.
5. Start the timer. As the test begins to work, you will see a reddish coloured liquid front moving across the membrane.
6. The result should be read after 10 minutes. Strong positive results may be observed sooner.
7. Do not interpret any test band appearing 15 minutes after the sample is dropped in the cassette.

Then, eliminate the components of the test and the sample according to the suitable procedure for potentially infectious waste.

RESULTS
The test result can be read visually or with the help of the BIOSYNEX Reader.

- Visual reading

IGFBP-1 AND AFP POSITIVE:

Presence of 3 distinct purple lines: A control line appears at the level of the C zone and two purple lines (even of weak intensity) appear at the level of A and B zones.
IGFBP-1 POSITIVE AND AFP NEGATIVE:

Presence of 2 distinct purple lines: A control line appears at the level of the C zone and one purple line (even of weak intensity) appears at the level of B zone.

AFP POSITIVE AND IGFBP-1 NEGATIVE:

Presence of 2 distinct purple lines: A control line appears at the level of the C zone and one purple line (even of weak intensity) appears at the level of A zone.

NEGATIVE:

Only one purple line appears at the level of the control line (C). No line appears at the level of the A and B zones.

INVALID:

No visible purple band at the level of control line C (whatever A and B band apparition). Results from a test with no control line must be discarded. Review the procedure and repeat it with a new test device. If the problem persists, contact your local distributor.

Reading with the reader

- The AMNIOQUICK DUO® test is compatible with the BIOSYNEX Reader, in combination with the SD card ‘AMNIOQUICK DUO®’.
- To read results with the reader, please refer to the reader Instructions for use. The barcode printed on the patient card should be scanned to identify the test lot and to check the compatibility of the SD card.

RESULTS INTERPRETATION

AMNIOQUICK® DUO® allows the detection of amniotic fluid in a sample of vaginal secretions through identification of two complementary markers whose concentration in amniotic fluid is very high: IGFBP-1 and AFP.

AMNIOQUICK® DUO® has been set up with a high IGFBP-1 threshold (10 ng/ml) in order to reduce this risk of false positive results due to presence in the vagina of phosphorylated IGFBP-1 originating from decidual cells of mature cervix. The positive predictive value (PPV) of IGFBP-1 of AMNIOQUICK® DUO® is therefore very high.

The concentration of AFP is fluctuating during pregnancy and it significantly decreases during the 3rd quarter of pregnancy. At the detection limit of the test (5 ng/ml), the positive predictive value (PPV) of AFP is very high beyond 37 weeks gestational age. In addition, the negative predictive value (NPV) of AFP before 37 weeks gestational age is very high.

The test has a sensitivity of 96.4%, a specificity of 87.5%, a positive predictive value of 97.8%, a negative predictive value of 82.3% and an accuracy of 95% in diagnosing ROM.

LIMITATIONS OF PROCEDURE

- As with all diagnostic tests, the test result must be considered with clinical findings.
- In case of significant quantities of blood in the sample of vaginal secretions, the result has to be interpreted cautiously.
- Presence of blood in the sample can lead to false positive AFP results especially before 39 weeks gestational age.
- Beyond 39 weeks gestational age, the decrease of AFP concentration in amniotic fluid might lead to false negative results.
- False negative results might appear when test is performed more than 12 hours after the leakage of amniotic fluid has stopped.
- The swab has to be diluted in extraction tube immediately after collection of sample. Then the tube can be kept for 6 hours maximum at room temperature or 4°C before running the test as proteases in vaginal secretions may alter IGFBP1 if stored longer than 6 hours.

PERFORMANCE S

Detection limit:

Detection limit is 10 ng/ml for IGFBP-1 and 5 ng/ml for AFP. Clinical performances of the test are depending on the selected reference method and on the level of suspicion of rupture in the selected population. The interpretation of the results according to above algorithm makes it possible to optimize the specificity of diagnostic of premature rupture of fetal membranes. It also allows to identify a population with undefined profile for which diagnostic should mainly be based on clinical symptoms.

Sensitivity and specificity

A study has been performed to evaluate the efficacy of AMNIOQUICK® Duo® in diagnosing rupture of membranes (ROM). A total of 100 women were enrolled into the study. Results have been interpreted according to the algorithm presented in table 1. Results are showed in below table.

Table 1: results interpretation algorithm

<table>
<thead>
<tr>
<th>RESULTS</th>
<th>AFP</th>
<th>IGFBP-1</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Rupture</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>No Rupture</td>
</tr>
</tbody>
</table>

Final clinical diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Rupture</th>
<th>No rupture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMNIOQUICK DUO®</td>
<td>81</td>
<td>2</td>
<td>83</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
<td>84</td>
<td>16</td>
<td>100</td>
</tr>
</tbody>
</table>

SYMBOLS

- Attention, see instructions for use

- For in vitro diagnostic use only

- Store between 2-30°C

- Tests per kit

- Expire

- Lot number

- Manufacturer

- Catalog number

- Extraction Buffer

Patent pending