Ref: 0552

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 (**I**nsulin-like **G**rowth **F**actor-**B**inding **P**rotein 1) and **AFP** (**A**lpha-**F**eto**P**rotein) 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 **P**remature **R**upture of **M**embranes 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 alkalinisation 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 subsequent 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 CE 0123
- Dropper bottles with extraction buffer DIL
- 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

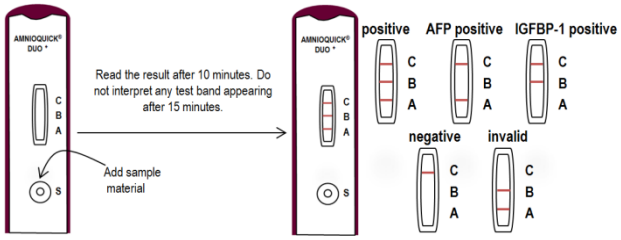
- For diagnostic *in-vitro* use only
- For best results, strictly follow the test procedure and storage instructions.
- Do not open the foil pouch until it reaches room temperature to prevent formation of condensation. Humidity and high temperature can affect results.
- Do not use the kit beyond the 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.
   Do not interpret any test band appearing 15 minutes after the sample is dropped in the cassette.
7. Then, eliminate the components of the test and the sample according to the suitable procedure for potentially infectious waste.

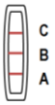


RESULTS

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

➢ **Visual reading**

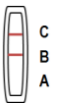
IGFBP-1 AND AFP POSITIVE:



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.

BIOSYNEX
12, rue Ettoré Bugatti – CS28006
67038 STRASBOURG Cedex – France
Tél. : +33 3 88 77 57 00
Fax : +33 3 59 81 21 74
info@biosynex.com
www.biosynex.com

IGFBP-1 POSITIVE AND AFP NEGATIVE:

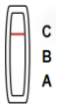


Presence of 2 distinct purple lines: A control line appears at the level of the C zone and one purple line **(even of weak intensity)** appears at the level of B zone.

AFP POSITIVE AND IGFBP-1 NEGATIVE:

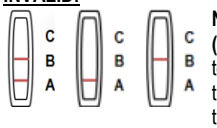
Presence of 2 distinct purple lines: A control line appears at the level of the C zone and one purple line **(even of weak intensity)** appears at the level of A zone.

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.

If the liquid front did not reach the control line level, repeat the procedure by adding one drop of diluted sample and 3 drops of diluent in the sample well of a new device.

➢ **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.

Taking into account the above features of those two markers and the detection limits, the results of the test should be interpreted according to the algorithm shown in the Table hereunder:

Table 1: results interpretation algorithm

	RESULTS				
AFP	Positive	Negative	Positive	Positive	Negative
IGFBP-1	Positive	Positive	Negative	Negative	Negative
Conclusion	RUPTURE		GA* ≥ 37 weeks	GA* < 37 weeks	NO RUPTURE
			RUPTURE	DOUBTFUL	

**Gestational Age*

QUALITY CONTROL

- Internal procedural controls are included in the test. A coloured line appearing in the control zone (C) ensures that sufficient specimen volume has been loaded and that the correct procedure has been followed by the operator.
- Good laboratory practices recommend the use of control materials to ensure proper kit performance. Control samples specific for this product are available separately.

LIMITATIONS OF PROCEDURE

- As with all diagnostic tests, the test result must be consistent 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

		Final clinical diagnosis		
		Rupture	No rupture	*Total*
AMNIOQUICK DUO+	Positive	81	2	83
	Negative	3	14	17
	Total	84	16	100

The test has a sensitivity of 96.4%, a specificity of 87.5%, a positive predictive value of 97.6%, a negative predictive value of 82.3% and an accuracy of 95% in diagnosing ROM.

Literature

1. *Seppala M., Ruoslahti E. Alpha fetoprotein in Amniotic fluid: An index of gestational age. Am. J. Obstet. Gynecol. 1972, November, 595-598*
2. *Rochelson BL, Richardson DA, Macri JN. Rapid assay: possible application in the diagnosis of premature rupture of the membranes. Obstet Gynecol 1983;62:414–418*
3. *Rutanen EM, Pekonen F, Kärkkainen T. Measurement of insulin-like growth factor binding protein-1 in cervical/vagonal secretions: comparison with the ROM-check membrane immunoassay in the diagnosis of ruptured fetal membranes. Clin Chim Acta 1993:214:73-81*
4. *Kishida et al. Diagnosis of premature rupture of the membranes in peterm patients, using an improved AFP kit: comparison with ROM-Check and/or nitrazine test. European Journal of Obstetrics and Gynecology and Reproductive Biology 1996, 69, 77-82*
5. *Rutanen et al. Evaluation of a rapid strip test for insulin-like growth binding protein-1 in the diagnosis of ruptured fetal membranes. Clinica Chimica Acta, 1996, 253, 91-101*
6. *Guibourdenche J, Luton D, André E, Noël M, Porquet D. Détection rapide de l'Insulin-like growth factor-binding protein-1 et de la fibronectine fœtale dans les sécrétions cervico-vaginales pour le diagnostic de la rupture prématurée des membranes. Ann Clin Biochem 1999 :36 :388-390*
7. *Gallot D., Sapin V. Menace d'accouchement prématuré et marqueurs de rupture prématurée des membranes : de la physiopathologie au diagnostic. Spectra Biologie, 2007, 161, 59-63*
8. *Rutanen et al. Radioimmunoassay of placenta protein 12 : levels in amniotic fluid, cold blood and serum of healthy adults pregnant women and patients with trophoblastic disease. Am. J. Obstet. Gynecol. 1982*
9. *Rutanen et al. Decidual transformation of human extrauterine mesenchymal cells is associated with the appearance of insulin-like growth factor binding protein-1. J. Clin. Endocreinal. Metab. 1992 72: 27-31*
10. *P. Ruanphoo and V. Phupong. Evaluation of the performance of the insulin-like growth factor-binding protein-1/alpha-fetoprotein test in diagnosing ruptured fetal membranes in pregnant women. Journal of Perinatology (2015), 1-3*

SYMBOLS

	Attention, see instructions for use	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Manufacturer
2°C 30°C	Store between 2-30°C		Do not reuse
Σ	Tests per kit	REF	Catalog number
	Expiry	DIL	Extraction Buffer

Patent pending

Version 06 BR 03/2015

BIOSYNEX
12, rue Ettoré Bugatti – CS28006
67038 STRASBOURG Cedex – France
Tél. : +33 3 88 77 57 00
Fax : +33 3 59 81 21 74
info@biosynex.com
www.biosynex.com