AmniSure® ROM (Rupture Of [fetal] Membranes) Test

INSTRUCTIONS FOR *IN VITRO* DIAGNOSTIC USE

INTENDED USE

The AmniSure ROM (Rupture Of [fetal] Membranes) Test is a rapid, non-instrumented, qualitative immunochromatographic test for the *in vitro* detection of amniotic fluid in vaginal discharge of pregnant women. The AmniSure ROM Test detects PAMG-1 protein marker of the amniotic fluid in vaginal discharge. The test is for use by health care professionals (*Rx ONLY) to aid in the detection of ROM in pregnant women reporting signs, symptoms, or complaints suggestive of ROM.

SUMMARY AND EXPLANATION OF THE TEST

The timely and accurate diagnosis of rupture of [fetal] membranes (ROM) is crucial because ROM may be associated with serious neonatal and maternal consequences. Failure to identify patients with ROM can result in the failure to intervene appropriately.

Conversely, the false diagnosis of ROM can lead to inappropriate interventions (e.g., hospitalization or induction of labor). Therefore, the correct and timely diagnosis of ROM is of crucial importance for the clinician.[1] Accurate diagnosis of [fetal] membranes rupture, however, remains a frequent clinical problem in obstetrics.[1-3]

Currently available tests have limitations and in some degree are invasive.[1] The AmniSure ROM Test is a rapid non-invasive strip test that can aid in the detection of ROM, providing rapid, easy-to-interpret and timely diagnosis. Consequently, measures can be taken in a timely manner to prevent complications. In clinical trials, *one* AmniSure ROM Test correlated with clinical diagnosis obtained through combined usage of *three* routinely used tests (Nitrazine, Ferning, and Pooling).

The AmniSure ROM Test kit is a self-contained test system providing qualitative results.

Health care professionals should use the test to evaluate patients with clinical signs/symptoms suggestive of [fetal] membranes rupture.

PRINCIPLE OF THE TEST

The AmniSure ROM Test does not require speculum examination that is used routinely today for ROM diagnosis. A sample of amniotic fluid (taken by vaginal swab) is placed into a vial with a solvent. The solvent extracts the sample from the swab for one minute, after which the swab is disposed. The AmniSure ROM Test strip, a lateral-flow device, is then dipped into the vial. The sample substance flows from the Pad Region of the strip to the Test Region. The test result is indicated visually by the presence of one, two, or no lines. The presence of only a control line indicates no membranes ruptured; two lines indicate there is a rupture. A test line only or no lines at all indicate an invalid result. See the "Test Procedure" section of these instructions for use for graphics depicting all possible test results.

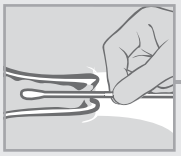
The AmniSure ROM Test uses the principles of immunochromatography to detect human PAMG-1 (placental alpha microglobulin-1) protein present in amniotic fluid of pregnant women. Placental Microglobulin was selected as a marker of [fetal] membranes rupture due to its unique characteristics, i.e. its high level in amniotic fluid, low level in blood, and extremely low background level (50-220 picogram/ml) in cervico-vaginal discharge when the [fetal] membranes are intact.

The AmniSure ROM Test employs highly sensitive monoclonal antibodies that detect even a minimum amount of the protein, which is present in cervico-vaginal discharge after the rupture of the [fetal] membranes. To minimize the frequency of false results, two monoclonal antibodies have been selected to set the sensitivity threshold of the AmniSure ROM Test at the optimal low level. This level allows the detection of extremely small quantities of amniotic fluid in vaginal discharge. Background concentration of PAMG-1 that uses this combination of monoclonal antibodies is around 50-220 picogram (i.e. 0.05-0.22 ng) per 1 ml of vaginal discharge. The sensitivity cut-off of the AmniSure ROM Test is 5 ng/ml, i.e. at least 20 times higher than the background concentration. This gap allowed increasing the accuracy of the AmniSure ROM Test.

During the test procedure, placental microglobulin from the sample sequentially binds to the monoclonal antibody conjugated with the label particles, and then to another monoclonal antibody, immobilized on an insoluble carrier. When conjugated antibodies come in contact with PAMG-1 on the Pad Region, they "catch" PAMG-1 and transport it to the Test Region. The Test Region of the test strip has antibodies immobilized on it. These antibodies "meet" PAMG-1 bound to conjugated antibodies flowing up from the Pad Region. This "meeting" immobilizes the system of PAMG-1/conjugated antibodies, resulting in a visible test line in the Test Region. This line is produced by gold dye attached to conjugated antibodies and indicates a Rupture Of [fetal] Membranes. The second control line is designed to indicate that the test is functioning properly. This line appears

Specimen Collection and Perpetration, Test Procedure, and Test Results

NOTE: You must follow all directions carefully to get an accurate reading of the results.
Do not use the Test earlier than 6 hours after the removal of any disinfectant solutions or medicines from the vagina.
Placenta previa and performing digital exams prior to sample collection can lead to inaccurate test results.

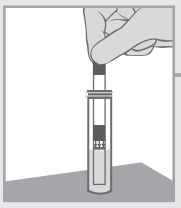


1. Take the solvent vial by its cap and shake well to make sure all liquid in the vial has dropped to the bottom. Place the solvent vial in an upright position and remove the cap.

2. To collect a sample from the surface of the vagina, use the sterile polyester swab provided with the AmniSure ROM Test. Remove the sterile swab from its package and follow the instructions on the packaging. The polyester tip of the swab should not touch anything prior to insertion into vagina. Hold the swab in the middle of its shaft and, while the patient is lying on their back, carefully insert the polyester tip of the swab into the vagina until the fingers contact the skin (no more than 2-3 inches or 5-7 cm deep). Withdraw the swab from the vagina **after one minute**.

3. After the swab has been removed from the vagina, immediately place the polyester tip into the provided solvent vial and rinse by rotating **for one minute**.

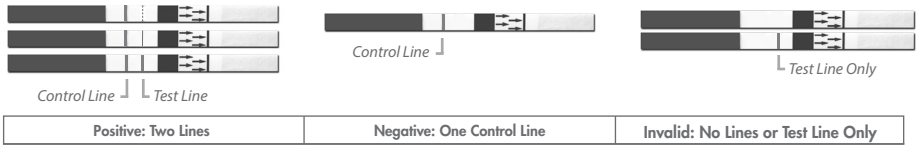
4. Remove the swab from the vial and dispose of it. Test the patient sample within 4 hours after collection. If the patient sample is not tested within 4 hours and sample storage is necessary, tightly close the sample vial and place in a refrigerator for an additional 2 hours. Do not test the sample after more than 6 hours have passed since sample collection.



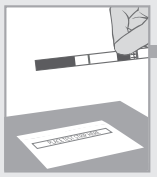
5. Tear open the foil pouch at the tear slits, and remove the AmniSure ROM Test strip.

6. Dip the white end of the test strip (marked with downward-facing arrows) into the solvent solution vial. Strong leakage of amniotic fluid may make the results visible early, while a very small leak will take the full **10 minutes**.

7. **Remove the test strip from the vial if two lines are clearly visible on the strip's test area (white space between the two blue 'blocks") or after 10 minutes sharp.** Do not read or interpret the results after 15 minutes have passed since inserting the test strip in the vial. Read the results by placing the test strip on a clean, dry and flat surface in a well-lit environment via either natural or fluorescent lighting. A positive result is indicated by two lines in the test area, while a negative result is indicated by the presence of a control line and no test line in the test area. Please note that the presence of no lines or only a test line indicates an invalid test result. DO NOT interpret this as a negative test result. Invalid results require a retest. To properly distinguish between the test and control lines, please see Step 8.



The intensity of the lines may vary; the test result is valid even if the lines are faint or uneven. Do not interpret the test result based on the intensity of the lines.



8. To ensure correct identification of test and control line locations, compare the test strip to the graphics on the outer kit bag as indicated. This will help confirm result interpretation.

LIMITATIONS OF THE TEST

- **Use AmniSure ROM Test results in conjunction with other clinical information.**
- Each test is a single-use disposable unit and cannot be reused.
- The AmniSure ROM Test results are qualitative. Make no quantitative interpretation based on the test results.
- When there is a significant presence of blood on the swab, the test can malfunction and is not recommended. In cases of only trace amounts of blood on the swab, the test still functions properly.
- In very rare cases when a sample is taken 12 hours or later after a rupture, a false-negative result may occur due to obstruction of the rupture by fetus or resealing of the amniotic sac.
- Test performance in patients without signs or symptoms of ROM is unknown.
- Failure to detect membrane rupture does not assure the absence of membrane rupture.
- Labor may occur spontaneously despite a negative test result.
- The performance of the AmniSure ROM Test has not been established in the presence of the following contaminants: anti-fungal creams or suppositories, K-Y® Jelly, Monistat® Yeast Infection Treatment, Baby Powder (Starch and Talc), Replens® Feminine Moisturizer, or Baby Oil.
- The performance of the AmniSure ROM Test has not been established in the presence of meconium in the amniotic fluid.

when anti-mouse IgG antibody "catches" the mouse antibody with gold dye. Gold dye gives the resulting line its color.

REAGENTS AND COMPONENTS

The AmniSure ROM Test kit includes the following components: 1) Instructions for use 2) AmniSure ROM Test strip in foil pouch with desiccant 3) Sterile polyester vaginal swabs 4) Plastic vial with solvent solution containing: 0.9% NaCl, 0.01% Triton X-100, 0.05% NaN_3.

STORAGE AND STABILITY

- Store the kit in a dry place at 4 to 25°C (40 to 77°F). DO NOT FREEZE.
- When stored in the foil pouch at the recommended temperature, the test is stable until the "Use By" date on the foil pouch.
- Use the AmniSure ROM Test within six (6) hours after removing from foil pouch.

QUALITY CONTROL

Each AmniSure ROM Test has built-in reagent and procedural controls to assure accurate reading of the results. The appearance of one or two lines in the test results area verifies the integrity of the test procedure. It is recommended to use external controls and to follow federal, state, and local guidelines for quality control requirements. Freeze-dried PAMG-1 protein is recommended for a positive external control and can be purchased from QIAGEN. Saline solution is recommended for negative external control.

Note: If separating the kit components prior to use, please record the AmniSure ROM Test kit lot numbers and/or specific strip, swab, and solvent lot numbers used for each test.

PRECAUTIONS AND WARNINGS

- A false-negative test may result in an inadequate level of care for newborns less than 37 weeks gestation if device is used in institutions other than those equipped to care for preterm infants (e.g. Level II-III nurseries).
- False-negative results can delay the diagnosis of rupture of membranes and can increase the risk of chorioamnionitis, oligohydramnios and fetal umbilical cord accident. **Negative** results alone may not rule-out membrane rupture.
- **Observe safety precautions when collecting, handling, and disposing of test samples.**
- Do not use damaged components of the test.
- Used test kits are biohazardous. Take proper precautions when handling/discarding used test kits.
- Do not use after the "Use By" date, which is printed on the foil pouch and on the box labeling.
- Do not reuse the test kit components.
- Do not bend or fold the test strip or the aluminum foil pouch with the test strip in it.
- Interrupted leakage with minimal residual fluid can lead to false-negative results.
- Until the diagnosis of membrane rupture is excluded, avoid digital cervical examination to prevent infection and shorten the latency period.
- For prescription use only. Federal law restricts this device to sale by or on the order of a physician.

EXPECTED VALUES

Leakage of amniotic fluid is indicative of the [fetal] membranes rupture in all women. Studies of placental alpha microglobulin-1 protein (PAMG-1) have established it as a marker of amniotic fluid.[4,5] Concentration of PAMG-1 in cervical and vaginal discharge of pregnant women without complications in pregnancy was measured and is ranged from 0.05 to 0.22 ng/ml. When vaginitis or non-significant admixture of blood serum is present, the background level of PAMG-1 can reach the maximum of 3 ng/ml. PAMG-1 concentrations in the amniotic fluid fall into 2,000-25,000 ng/ml range. Clinically significant leakage of amniotic fluid increases PAMG-1 concentration in cervico-vaginal discharge by a factor of thousands. The sensitivity threshold of the AmniSure ROM Test is set by a factor of 20 above the background level of PAMG-1 (The AmniSure ROM Test detects 5-7 ng/ml of PAMG-1).

PERFORMANCE CHARACTERISTICS

The clinical performance of the AmniSure ROM Test was determined by three studies where it was compared to clinical diagnosis provided by a combination of routinely used Nitrazine, Ferning and Pooling tests.[1,7] The diagnosis was set when two out of three control tests gave identical results (2-out-of-3 method). A total of 432 patients were evaluated at different sites. Of these patients, 108 were from women below 34 weeks gestation; the majority of those 108 (70%) were above 24 weeks. Patient gestational age ranged from 11-41 weeks. Exclusion criteria included active vaginal bleeding from any source and placenta previa. Statistical analysis is available for these 432 cases. Relative to clinical diagnoses determined by routine clinical tests, the AmniSure ROM Test's sensitivity, specificity, and 95% Confidence Intervals (CI) were estimated as follows:

Site	# of Patients	Sensitivity (95% CI)	Specificity (95% CI)
Sharp[1]	203	98.9% (93.2-99.9%)	100.0% (95.9-100%)
Yale/Seoul[7]	183	98.7% (95.1-99.8%)	87.5% (66.5-96.7%)
Wesley	46	100.00% (83.4-100%)	100.00% (80.8-100%)
Total (gestational age 11-41 weeks)	432	**98.9% (96.6-99.7%)**	**98.1% (94.1-99.5%)**

Pooled Data:		
	<34 weeks[1,7] (95% CI)	≥34 weeks (95% CI)
Sensitivity	98.3% (90.9-99.9%)	99.07% (96.8-99.8%)
Specificity	95.9% (86.0-99.5%)	99.07% (95.4-99.9%)

Parallel to Nitrazine, Ferning, and Pooling tests, admitted women were tested with the AmniSure ROM Test.

INTERFERENCE STUDIES

Vaginal infections or urine do not interfere with the results of the AmniSure ROM Test. Detailed research and analysis showed that PAMG-1 concentration in vaginal exudates during infections never exceeds the level of 3 ng/ml. The AmniSure ROM Test's sensitivity level is 5 ng/ml, excluding any interferences resulting from infections. Concentration of PAMG-1 in sperm was found not to exceed 4 ng/ml. Concentration of sperm PAMG-1 in vaginal discharge is even lower due to four-time dilution effect during testing. Therefore, during the development of the AmniSure ROM Test and during clinical trials, there was no interference of sperm factor in the results.

The same is true for urine. Fifteen samples of urine were studied for PAMG-1 concentration in it, using ELISA. Sensitivity of ELISA was 0.5 ng of PAMG-1 per 1 ml of solution. Parallel to that, the AmniSure ROM Test was also used to detect PAMG-1 in urine. Samples have been obtained from pregnant women at 25-40 weeks of pregnancy. Both methods gave negative results: PAMG-1 has not been found.

CROSS REACTIVITY

The specificity of monoclonal antibodies used in the AmniSure ROM Test was tested by studying their cross-reactive binding to proteins: alpha-2-microglobulin of fertility, human chorionic gonadotropin, trophoblastic beta-1-glycoprotein, human placental lactogen, alpha-fetoprotein, human serum albumen, and some IGFBP proteins. Monoclonal antibodies used in the AmniSure ROM Test were not cross-reactive to other proteins, except that antibody used in the Test line was found cross-reactive to IGFBP-3 protein in ELISA. It was shown that concentration of IGFBP-3 in vaginal discharge of pregnant women reaches 680 ng/ml, but this concentration does not impact the sensitivity of the AmniSure ROM Test to PAMG-1.

STABILITY OF RESULTS

The AmniSure ROM Test's results can be read if two stripes are clearly visible in the vial or at 10 minutes sharp after the Test strip is dipped into the vial. Stability tests were conducted where a lot containing one thousand the AmniSure ROM Test kits has been studied. Purified PAMG-1 has been used in concentrations of 10 ng and 5 ng of PAMG-1 per 1 ml of physiologic saline solution.

The duration/stability of results was also measured. After the result became visible (lines appeared in the test region), it remained stable for at least 5 min. This kind of stability is observed when PAMG-1 concentration is very small (5-10 ng/ml). When the concentration is higher, the lines remain stable for hours. In using the AmniSure ROM Test, it is recommended that the results not be read or interpreted after 15 minutes are passed after the Test strip is dipped into the vial.

BIBLIOGRAPHY

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2. *Lockwood CJ et al. Fetal membrane rupture is associated with the presence of insulin-like growth factor-binding protein-1 in vaginal secretions. Am. J. Obstet. Gynecol. 1994 Jul; 171(1):146-50.*
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4. *Petrunin DD et al. Immunochemical identification of organ specific human placental alpha1-globulin and its concentration in amniotic fluid. Akush Ginekol (Mosk). 1977 Jan; (1):62-4.*
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6. *Chen FC, Dudenhausen JW. Comparison of two rapid strip tests based on IGFBP-1 and PAMG-1 for the detection of amniotic fluid. Am J Perinatol. 2008 Apr; 25(4):243-6.*
7. *Lee SE et al. Measurement of placental alpha-microglobulin-1 in cervicovaginal discharge to diagnose rupture of membranes Obstet Gynecol. 2007 Mar; 109(3):634-40.*

GLOSSARY OF SYMBOLS

Graphic Symbol	Symbol Title / Number / Description	Graphic Symbol	Symbol Title / Number / Description
IVD	In vitro diagnostic medical device / 5.5.1 / Indicates a medical device that is intended to be used as an in vitro diagnostic medical device**		Consult instructions for use or consult electronic instructions for use / 5.4.3 / Indicates the need for the user to consult the instructions for use**
UDI	Unique device identifier / 5.7.10 / Indicates a carrier that contains unique device identifier information**		Do not use if package is damaged and consult instructions for use / 5.2.8 / Indicates that a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for additional information**
RX ONLY	RX Only / N/A / Federal law restricts this device to sale by or on the order of a physician*		
REF	Catalog number / 5.1.6 / Indicates the manufacturer's catalogue number so that the medical device can be identified**		Contains sufficient for <n> tests / 5.5.5 / Indicates the total number of tests that can be performed with the medical device**
LOT	Batch code / 5.1.5 / Indicates the manufacturer's batch code so that the batch or lot can be identified**		Temperature limit / 5.3.7 / Indicates the temperature limits to which the medical device can be safely exposed**
	Caution / 5.4.4 / Indicates that caution is necessary when operating the device or control close to where the symbol is placed, or that the current situation needs operator awareness or operator action in order to avoid undesirable consequences**		Use-by date / 5.1.4 / Indicates the date after which the medical device is not to be used**
	Do not re-use / 5.4.2 / Indicates a medical device that is intended for one single use only**		Manufacturer / 5.1.1 / Indicates the medical device manufacturer**

*Regulation: 21 CFR 809.10 (a)(4)
**Regulation: ISO 15223-1: Medical devices – Symbols to be used with information to be supplied by the manufacturer

PATENT AND TRADEMARK INFORMATION

QIAGEN®, Sample to Insight®, AmniSure®. (QIAGEN Group)

K-Y® Jelly (Owned by Reckitt Benckiser, Slough, England)

Monistat® Yeast Infection Treatment (Owned by Insight Pharmaceuticals, White Plains, New York)

Replens® Feminine Moisturizer (Owned by Church and Dwight Co., Inc., Ewing, New Jersey)

The AmniSure ROM Test and its use are covered by one or more of the following patents granted or licensed to QIAGEN and/or its subsidiaries; U.S. Patent 7,709,272, corresponding foreign patents and other patents pending.

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IVD

Ordering www.qiagen.com/shop | Technical Support support.qiagen.com | Website www.qiagen.com

DOCUMENT REVISION HISTORY

Revision	Release Date	Change
01	Mar. 2015	Initial Release
02	Apr. 2016	Updated timing on testing and reading
03	Jan. 2017	Updated to accommodate French translations
04	Jun. 2019	Reorgainzed instructions to have clarity and better definition of instructions
05	Aug.2021	Update to "Sample to Insight" Branding
06	Jan. 2022	Added contact information to back cover
07	Nov. 2024	Reorgainzed instructions to have clarity and better definition of instructions