I. **AMINOTEST (YELLOW NITRAZINE SWAB)**

1. **PURPOSE**
   
   Nitrazine Yellow is used to measure the pH of amniotic fluid. Rupture of the amniotic membrane can result in small volumes of amniotic fluid leaking into the upper vagina. The presence of amniotic fluid tends to elevate the pH of the upper vagina. Detection of this pH increase using a pH indicator dye has been shown to assist in determining the presence of amniotic fluid.

2. **PRINCIPLE**
   
   A swab impregnated with Nitrazine yellow dye is brought in contact with the upper vagina. The swab absorbs fluid associated with the tissue and the dye develops a color which correlates with the pH of the absorbed fluid over a range of pH 5.5 - pH 7.5.

   Amniotic fluid has a neutral pH while the pH of the upper vagina is normally acidic. A pH of 6.5 or higher in the upper vagina is consistent with leakage of amniotic fluid.

3. **SUPPLIES and REAGENT**
   
   Pro-Lab AmnioTest Swabs impregnated with Nitrazine yellow dye. The swabs are packaged in individual sleeves. Swabs are sterilized by gamma eradication and are sterile until opened.

4. **STORAGE**
   
   Store swabs at room temperature (15-30 C). When stored as stated, the swabs are stable until the expiration date on the label.

5. **PRECAUTIONS**
   
   1. Testing personnel should always wear gloves when performing this test.

   2. Pro-Lab AmnioTest is intended for In Vitro use only.

   3. Do not use AmnioTest after expiration date shown on the product label.
4. Because this test is visually read, it should not be used by those who are colorblind or visually impaired.

5. Following contact with the vagina, swabs should be considered potentially infectious and precautions appropriate with microbiological hazards (universal precautions) should be followed.

6. Do not reuse swabs.

6. PATIENT PREPARATION

1. Physician prepares patient for a speculum examination.

2. A swab is removed from its protective sleeve. DO NOT touch the tip of the swab or allow it to come into contact with any liquid or other substance which might affect pH.

3. Part the labia exposing the cervix and carefully insert the swab into the vagina. Do not allow the swab to come into contact with vaginal tissue during entry.

4. Allow first and only contact of the Amniotest swab tip to occur with upper vaginal tissue (posterior vaginal fornix and external cervical os).

5. Allow the tip to remain in contact with upper vaginal tissue for about 15 seconds.

6. Carefully remove the swab and immediately examine the color of the tip for pH interpretation.

7. Compare results with sample colors on the AmnioTest Color card.

7. INTERPRETATION OF RESULTS

The color of the tip of the AmnioTest swab after use should be compared to the sample colors of the AmnioTest Color card.
Color | Approximate pH value | Indication | Consistent With:
--- | --- | --- | ---
Yellow | not applicable | Fresh swabs |
Yellow-gold | 5.0 | Intact Amniotic Membrane |
Yellow-olive | 5.5 | Intact Amniotic Membrane |
Olive | 6.0 | Intact Amniotic Membrane |
Dark green | 6.5 | Possible Ruptured Amniotic Membrane |
Dark Blue-green | 7.0 | Possible Ruptured Amniotic Membrane |
Navy Blue | 7.5 or higher | Possible Ruptured Amniotic Membrane |

### 8. QUALITY CONTROL POLICY

1. Each AmnioTest shipment lot is quality checked by the Point-Of-Care-Testing Department staff upon receiving supplies from L&D.

2. 2 levels of pH standard buffers 5.0 and 7.0 are used to span the linearity of the readable ranges. Store buffers at room temperature. Buffers are stable until the expiration date on the bottles.

3. If each of the pH values written on the color card corresponds to the pH of each of the buffer solutions then the swab is performing as expected and is acceptable for patient testing. Repeat the test if any of the pH colors on the swab does not match with the corresponding pH on the color card.

4. Acceptable quality checked swabs can be released to L&D upon request.
5. If repeated test fails to yield acceptable results matching to the color card, reject the shipment and notify manufacturer to address issue. Notify L&D to order a new shipment lot.

6. Quality Checks and room temperature readings are recorded on the Quality Control log.

9. **LIMITATIONS OF THE PROCEDURE**

   Antibiotic therapy or infections of the vagina can lead to elevated vaginal pH resulting in a false interpretation of determining the presence of amniotic fluid. Where doubt exists, standard microbiological testing should be employed to exclude infection.

10. **RESULT REPORTING**

    AmnioTest result is reported in conjunction with the Fern Test result. See below for details. A nursing staff records pH and microscopic results on the log, indicating name of the physician who performed these tests.

II **MICROSCOPY EXAMINATION OF FERN PATTERN OF AMNIOTIC FLUID**

1. **PRINCIPLE**

   The Fern Test, used with the Nitrazine test, detects the leakage of amniotic fluid from the membranes surrounding the fetus during pregnancy. Premature rupture of the membranes may lead to fetal infection and subsequent mortality. The risk may be eliminated by induction of labor.

2. **SPECIMEN REQUIREMENTS**

   1. Vaginal secretion from the posterior vaginal pool collected with a sterile swab.
   2. Do not touch the mucus plug in the cervix.
   3. After collection, immediately rub the swab against a glass slide, creating a very thin smear.
   4. Allow slide to dry.

3. **INTERFERENCES**

   1. False positive results may occur from specimens contaminated with blood, urine, or cervical mucus.
2. False negative results may occur from prolonged rupture of the membranes (longer than 24 hours).
3. False negative results may occur if only a small volume of fluid has leaked.

4. REFERENCE RANGE

N/A

5. ALERT VALUES

N/A

6. QUALITY CONTROL

N/A

7. PROCEDURE

1. Using a microscope, examine the dried smear under low power without a cover slip.
2. If present, the amniotic fluid crystallizes to form a fern-like pattern due to the relative concentrations of sodium chloride, proteins, and carbohydrates in the fluid.
3. If ferning is difficult to locate, examine all fields on the slide thoroughly.

8. REAGENTS

1. Sterile glass slide.
2. Sterile swab.
3. A well maintained microscope

9. RESULTS REPORTING (AmnioTest and Fern Test results)

1. The Fern Test should be performed in conjunction with the AmnioTest Swab Test.
2. If the AmnioTest Swab Test and Fern Test are positive, probable membrane rupture has occurred.
3. If the AmnioTest Swab Test is negative but the Fern Test is positive, there is probable rupture of the membranes due to the Fern Tests greater specificity.
4. If the AmnioTest Swab Test is positive but the Fern Test is negative, a second specimen should be collected and tested.
5. Assisting nursing staff records both AmnioTest and Fern Test results as indicated by the physician who performed the testing. The results are record on a log, indicating the results,
name of the physician who performed the tests and the nurse who record the results. Patient label (MRN#, BD and patient name) is pasted on to the log following correct identification of the patient.

6. Results are entered into the Hospital Information System (EPIC) as soon as the procedure is completed.

10. Policy

1. Physician competency with AmnioTest is demonstrated with the on-line training and completion of a set of competency questions, including color blindness testing. Reading of the pH color is considered a process similar to pH Nitrazine paper which is a waived test category. Annual competency consists of observation of routine work by the nursing staff for each procedure and completion of the annual competency test.

2. Physician competency with specimen collection of amniotic fluid is part of the Ongoing Professional Evaluation (OPPE) which is required by the hospital standard MS.08.01.03. Physicians are required to complete the initial and annual on-line competency (www.MTStraining.com) on Vaginal Wet Prep and Fern Testing. College of American Pathologist Proficiency Testing is subscribed for physician participation twice per year. Results are graded and corrective actions are required with unsatisfactory performance. Refer to the file regarding Governance of Provider Performed Microscopy (PPMP) Competency and Proficiency Testing.

11. References

1.) Apothecon, Princeton, NJ, Nitrazine Paper, p0083-00, p—0084-00 52520.
5.) AmnioTest product insert, ProLab Diagnostics, Round Rock, Texas, 2012 03
6.) Addison, Lois Anne. Laboratory Medicine, July, 1999. P.451