TITLE: FERN TEST – AMNIOTIC FLUID CRYSTALLIZATION TEST POLICY AND PROCEDURE

EFFECTIVE DATE: October 10, 2005 **SOP #** POC.038

APPROVED BY: **PAGE** 1 of 6

Patricia Tsang, MD MBA / Laboratory Chairperson Date _____

Cyril D'Cruz, MD / Laboratory Vice Chair Date _____

Maricar Eckman, BSMT / Point of Care Coordinator Date _____

REVIEWED BY:

Date	Medical Director	Date	Supervisor

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ATTACHMENTS:

Attachment A: Fern Testing Certification of Competency

PURPOSE:

To provide instructions to perform the fern test which enables the provider to detect premature leakage of amniotic fluid from fetal membranes in minimal time.

QUALIFICATIONS:

1.0 Personnel:

1.1 The following personnel are authorized to perform Fern testing when credentialed as competent:

- **Physicians (OB/GYN)**
- **Midwives**

1.2 The Director of the Nurse Midwifery Practice will be responsible for the training and supervision of Fern testing performed by the midwives.

1.3 The Chair of the OB/GYN department will be responsible for credentialing physicians for fern testing.

1.4 The Laboratory Point-of-Care Coordinator (or designee) will be responsible for the oversight of the Point-of-Care testing at each location within NBIMC.

2.0 Certification:

2.1 Documentation of policy and procedure review by the physicians / midwives performing Fern testing.

2.2 Documentation of training performed by the Director of the Nurse Midwifery Practice, who is a certified trainer **(See Attachment A).**

POLICY:

1.0 Fern testing is a part of the OB/GYN physicians' credentialing process and is monitored by the Chair of the OB/GYN department.

2.0 Results are written in the patient's chart by the practitioner performing the test. **Date and time of test**, **test result**, and **initials** of the person performing the test are included in the chart.

PRINCIPLE:

The fern test is based on the ability of amniotic fluid to form a fern pattern when air-dried on a glass slide; this phenomenon in part is due to the fluid's protein and sodium chloride content. A positive test shows the presence of fern-like patterns characteristic of amniotic fluid crystals.

REAGENTS AND SUPPLIES:

*** No reagents required**
Sterile vaginal speculum
Sterile cotton-tipped swab or pipette
Microscope slides
Microscope
Gloves

SPECIMEN:

A vaginal swab obtained from the posterior vaginal pool collected with a sterile swab and placed immediately on a glass slide for microscopic interpretation.

SPECIMEN COLLECTION AND HANDLING:

1.0 Standard precautions must be observed at all times when handling body fluids.

2.0 Position patient in the dorsal lithotomy position.

3.0 Avoid the use of any lubricants or antiseptics.

4.0 Place sterile speculum into vaginal vault.

5.0 Obtain a sample of vaginal secretions from the posterior vaginal pool, using a sterile swab or pipette.

6.0 Be sure not to touch the mucus plug.

7.0 Place fluid on glass slide, spreading specimen evenly so that a **thin smear** is formed. **Do not place a cover slip on the specimen.**

8.0 Allow slide to **air-dry**. This will require about 5 minutes.

9.0 **Do not apply heat.**

TESTING PROCEDURE:

1.0 Examine the slide under low power (10X).

2.0 Look for fern-like crystals. **Ferning** will be easily seen in most fields if the test is positive.

3.0 Report results as either:

Fern Test positive for amniotic fluid
Fern Test negative for amniotic fluid

4.0 Record patient results in patient's chart with signature or initials of person performing the test, date and time of procedure.

LIMITATIONS OF THE METHOD:

1.0 False positive results may occur from specimens contaminated with blood, urine, cervical mucus, soaps, or infection.

2.0 False negative results may occur from prolonged rupture of the membranes (longer than 24 hours).

3.0 False negative results may occur if only a small volume of fluid has leaked.

EXPECTED RESULTS:

1.0 Depending on the gestational age, traces of amniotic fluid in vaginal secretions may be non-detectable.

2.0 Cocaine actively increases myometrial contractile activity. Recent literature states women with recent cocaine use present with ruptured membranes at an earlier gestational age. However, their latency period between rupture of membranes and delivery is longer than non-cocaine users.

3.0 In pregnancy, bacterial vaginosis has been associated with premature birth, premature rupture of membranes, and chorioamnionitis. In fact, *Gardnerella vaginalis* is a common isolate in postpartum and postabortal fevers. It can cause fatal and non-fatal septicemia and fetal scalp monitor infections in neonates.

PROFICIENCY TESTING PROCEDURE:

1.0 Proficiency testing is provided by the University of Washington through an on-line PPM competency challenge program, approved by CAP, the College of American Pathologists.

2.0 Physicians and non-physicians who perform this test may participate in this program, at the discretion of the Chair of the OB/GYN department.

3.0 The passing grade for this on-line program is 80%.

4.0 Incorrect results will be investigated and retraining performed if necessary.

MICROSCOPE PREVENTATIVE MAINTENANCE:

1.0 Proper care and preventative maintenance should be performed **daily or after use** in order to ensure optimum performance.

2.0 A thorough bi-annual cleaning by a technical representative is required.

3.0 The microscopic maintenance will be performed concurrently with the laboratory maintenance schedule, provided by an outside service.

4.0 **Maintenance procedure:**

4.1 The microscope should be kept covered when not in use.
4.2 Keep surfaces free from dust.
4.3 Clean objective lenses, eyepieces and condenser daily, or after use.

- Use a high qulity lens paper that has been dampened with an approved lense cleaner. Do not use Xylene or other chemicals.
- Dry lens paper can scratach the lenses
- DO NOT USE Kimwipes, commercial tissue, or gauze.
- The 10X and 45X objective must be kept oil-free at all times. Oil can impose permanent damage to the objective.
- Preventive Maintenance is scheduled by lab administrator with appropriate company twice a year.

INFECTION CONTROL:

Handle and dispose of all materials coming in contact with blood according to standard precautions. Any spills or leakage must be cleaned with a disinfectant (10% bleach).

SAFETY:

Sharps precautions must be utilized or followed at all times.

REFERENCES:

Addison, Lois Anne. Laboratory Medicine, July 1999.

Fern Test CIOLA Guide to Simple Laboratory Testing, February 1998.

ORIGINAL DATE:
October 2005

INITIATED BY:
Nellie Gillman, POCC

REVISED DATE:
February 2012

TITLE: pH, AMNIOTIC FLUID BY AMNIOTEST (Nitrazine Yellow Swabs) – POLICY and PROCEDURE

EFFECTIVE DATE:	June 1, 2006	**SOP #**	POC.040
APPROVED BY:		**PAGE**	1 of 7

Date
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ATTACHMENTS:

Attachment A: Initial Competency for AmnioTest
Attachment B: AmnioTest Quality Control Log

PURPOSE:

AmnioTest is a convenient swab screening system intended as an aid to detect small quantities of amniotic fluid in vaginal secretions, based on the difference in pH between vaginal secretions and amniotic fluid. The presence of amniotic fluid tends to elevate the pH of the upper vagina. It is used in conjunction with the Fern Test to help detect the rupture of amniotic membranes in pregnant women. Premature rupture of the membranes before onset of labor may lead to fetal infection and subsequent mortality. The risk is largely eliminated by induction of labor.

PRINCIPLE OF THE PROCEDURE:

A swab impregnated with nitrazine yellow dye is brought into 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 the range of pH 5.5 to 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.

QUALIFICATIONS:

1.0 Personnel:

1.1 The following personnel are authorized to perform AmnioTest swab testing when trained and validated as competent:

- **Physicians (OB/GYN)**
- **Midwives**

1.2 The Director of the Nurse Midwifery Practice will be responsible for the training and supervision of AmnioTest swab testing performed by the midwives.

1.3 The Chair of the OB/GYN department will be responsible for assuring compliance with the AmnioTest swab testing policy and procedure.

1.4 The Laboratory Point-of-Care Coordinator (or designee) will be responsible for the oversight of the Point-of-Care testing at each location within NBIMC.

2.0 Certification:

2.1 Documentation of policy and procedure review by the physicians / midwives performing the AmnioTest swab test.

2.2 Documentation of training performed by the Director of the Nurse Midwifery Practice, who is a certified trainer **(See Attachment A).**

POLICY:

1.0 AmnioTest swab testing is being made a part of the OB/GYN physicians' credentialing process. As part of the credentialing process, any physician performing the test must be familiar with the policy and procedure.

2.0 The midwives and residents are required to perform, bi-annually, the CAP (College of American Pathologists) Proficiency testing. Testing will be performed by a different user each time a CAP sample is received.

3.0 Results are written in the patient's chart by the practitioner performing the test. **Date and time of test**, **test result**, and **initials** of the person performing the test are included in the chart.

REAGENTS:

1.0 **AmnioTest swab** – disposable swabs impregnated with nitrazine yellow dye and packaged in individual sleeves.

- Swabs are sterilized by gamma radiation and are sterile until opened.
- Do not reuse swabs
- AmnioTest swabs intended for In Vitro use only.
- Do not use AmnioTest after expiry date on product label.

2.0 Storage – sleeves should be stored at room temperature (15 - 30°D). Product is stable until the expiry date on label when sealed in individual sleeves.

3.0 **Pro-Lab Diagnostics Buffer Solution** – 2 levels of buffers to show positive and negative results.

QUALITY CONTROL TESTING:

1.0 Two levels of quality control (QC) will be performed by Point of Care Coordinator or designee with every new shipment of AmnioTest or with each new lot number sent for lot-to-lot comparison. **(See Attachment B).**

2.0 2 levels of buffers with a known pH will be used to perform quality control testing to show the color reactions of the positive and negative results on the NitraTest paper.

3.0 **Procedure:**

3.1 Remove one AmnioTest swab from its sleeve.

3.2 Wet the swab's tip with 3-4 drops of buffer solution.

3.3 Immediately compare the color developed on the swab tip to the closest matching color on the AmnioTest Color card.

3.4 If the pH value written next to the color selected on the card corresponds to the pH of the buffer solution, then the swab is performing as expected.

3.5 If the AmnioTest swab does not produce the expected color reaction, repeat QC test with a fresh swab.

3.6 If QC fails a second time, do not use the AmnioTest swab for patient testing. First check the pH of buffer used for QC with a pH meter. Do not use the remaining swabs in the kit if the QC is deemed accurate with the pH meter.

SPECIMEN COLLECTION AND HANDLING:

1.0 Vaginal secretions from the posterior vaginal pool.

2.0 Do not touch the AmnioTest swab to the mucus plug in the cervix.

3.0 Test sample immediately after collection.

TESTING PROCEDURE:

1.0 Remove a swab 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.

2.0 Use sterile exam gloves. No lubrication should be used on exam gloves.

3.0 Part the labia exposing the cervix and carefully insert the swab into the vagina.

4.0 Do not allow the swab to come into contact with vaginal tissue during entry.

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

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

7.0 Note the color and turbidity of the fluid. Document any possible contamination, which invalidates the test.

8.0 Immediately match the strip color with the closest color on the color chart. Avoid touching the chart. Clean with sani-cloth or bleach wipes if accidentally contaminated.

9.0 **Record result** in patient's chart, with **date, time** and **initials** of the person performing the test.

REFERENCE RANGES:

1.0 Normal vaginal secretions have a pH of 4.5 – 6.0.

2.0 **Amniotic fluid** has a pH of 6.5 – 7.5. When membranes rupture, the amniotic fluid leaks into the vagina and raises the pH of the vaginal secretions.

INTERPRETATION:

1.0 **Results:**

Color Range	Approx pH	Interpretation
Yellow-Olive	**pH 5.0 – 6.0**	**Negative for amniotic fluid**

Olive-green to Blue-black	**pH 6.5 – 7.5 or higher**	**Positive for amniotic fluid**

2.0 **Positive AmnioTest swab, Positive Pooling,** and **positive Fern test** – probable membrane rupture.

3.0 **Negative AmnioTest swab and positive Fern test** – probable membrane rupture. Fern test has greater specificity.

4.0 **Positive AmnioTest swab and negative Fern test** – not diagnostic.

RESULT REPORTING:

1.0 The result is reported in the patient's chart as:

- Negative for amniotic fluid, or
- Positive for amniotic fluid

2.0 Result must be accompanied by the **initials** of the person performing the test, and the **date and time of test performance**.

LIMITATIONS OF THE METHOD:

1.0 The PRO-LAB AmnioTest is designed to be used by qualified medical professionals and is intended as an aid to professional diagnosis.

2.0 AmnioTest can only indicate a change in pH value and should be used only as indicated in the test procedure described above.

3.0 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.

4.0 Blood, mucous, alkaline antiseptics, vaginal infections and alkaline urine – may cause false positives for AmnioTest swab test results.

5.0 Possible presence of any of these contaminants makes test inaccurate and voids results.

6.0 The AmnioTest will be correlated to clinical presentation of the patient. If the result does not correlate to the patient's symptoms, refer to physician for further evaluation.

PROFICIENCY TESTING PROCEDURE:

1.0 Proficiency testing is performed bi-annually through the College of American Pathologists (CAP).

2.0 The blind samples sent will be rotated for testing by all the non-physician personnel certified to perform **AmnioTest swab** testing.

3.0 Personnel who performed the Proficiency survey and did not test correctly will be asked to perform testing on buffer solutions to verify competency.

4.0 Competency assessment of licensed physicians performing this test will be established and monitored by the credentialing process of the medical staff.

INFECTION CONTROL:

Handle and dispose of all materials coming in contact with blood according to universal precautions. Any spills or leakage must be cleaned with a disinfectant (10% bleach).

SAFETY:

Sharps precautions must be utilized or followed at all times.

REFERENCES:

PRO-LAB Diagnostics AmnioTest Package Insert, Rev. 2001

ORIGINAL DATE:

May 2006

INITIATED BY:

Initiated by Nellie Gillman, POCC

REVISED DATE:

February 2012

TITLE: WET MOUNT PREPARATION POLICY AND PROCEDURE

EFFECTIVE DATE: August 9, 2006 **SOP #** POC.043

APPROVED BY: **PAGE** 1 of 8

Patricia Tsang, MD MBA / Laboratory Chairperson Date _____

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REVIEWED BY:

Date	Medical Director	Date	Supervisor

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ATTACHMENTS:

Attachment A: List of Approved Point of Care Tests
Attachment B: 10% Potassium Hydroxide Reagent Droppers Package Insert

PURPOSE:

Vaginitis is one of the most common complaints of women seeking medical treatment. Approximately ninety percent (90%) of the cases are associated with infections caused by ***Bacterial Vaginosis, Candida sp. or Trichomonas.*** In conjunction with the patient's history and physical examination, the wet prep procedure is a valuable aid in the diagnosis of the etiologic agent.

This procedure provides instructions for performing microscopic examination for the presence of these agents in saline suspension of vaginal secretions, as specified in the guideline established by the College of American Pathologists (CAP). It is also in compliance with the Clinical Laboratory Improvement Amendment (CLIA).

QUALIFICATIONS:

1.0 Personnel:

1.1 The following personnel are authorized to perform wet mount testing if it is within the scope of their medical specialties and when credentialed as competent:

- **Attending Physicians**
- **Resident Physicians**

1.2 The Chair of each department will be responsible for the training program approved by the lab and supervision of wet mount prep testing performed by the physicians and residents.

1.3 If applicable, the department Chair will be responsible for credentialing physicians for wet mount testing, when it is added to the physicians' credentialing process.

1.4 The Laboratory Point-of-Care Coordinator (or designee) will be responsible for the oversight of the Point-of-Care testing at each location within NBIMC.

2.0 Departments qualified for wet mount testing:

2.1 Certain departments/sites are authorized by the Laboratory for testing if the test falls within the scope of their particular specialty practice and upon satisfying all compliance requirements:

See list of Approved Point of Care Tests (See Attachment A)

3.0 Certification:

3.1 Documentation of policy and procedure review by the physicians/residents performing wet mount testing.

3.2 Documentation of all required training on Signature sheets and through print-outs of the on-line competency tests performed by each physician or resident.

POLICY:

1.0 Until wet mount prep testing is added to the physicians' credentialing process, the training for the attending physicians will require them to read and be familiar with the policy and procedure and to sign the Signature log to document this.

2.0 The initial training for attending physicians will include enrolling in an on-line proficiency test and passing with an 80% score.

3.0 Resident physicians are required to document that they have read and are familiar with the policy and procedure and will have to enroll in the on-line competency program, annually.

4.0 KOH lyses epithelial cells in about 5 minutes and allows easier microscopic visualization of Candidal hyphae. 10% KOH is a caustic and has a potential for injury.

REAGENTS AND EQUIPMENT:

Equipment:

- Microscope with 10x and 40x objective lenses

1.0 **Materials:**

- Test tubes (10 x 75 mm)
- Sterile swabs
- Glass microscope slides
- Glass cover slips 22 mm sq.
- Disposable plastic pipettes
- Microscope lens cleaner
- Lens paper
- A puncture proof disposal container
- An EPA approved disinfectant

2.0 **Reagents:**

- Normal saline, 0.85% sodium chloride in water
- 10% Potassium Hydroxide (KOH)

STORAGE:

1.0 All reagents are to be stored at room temperature.

2.0 No reagents are to be used past their expiration date.

MAINTENANCE:

1.0 **Daily**:

- Clean objective lenses, eyepieces and condenser daily. Use high quality paper moistened with an approved lens cleaner to clean objectives and eyepieces.

Note: DO NOT USE organic solvents, Kimwipes, paper towel, or gauze to clean lenses or eyepieces.

- Clean work surfaces daily or when contaminated, with an EPA approved disinfectant.

2.0 **Bi-Annually:**

- The microscope must have a bi-annual preventive maintenance by a qualified service agent.
- This will be performed in the laboratory and each department will be notified when to bring their microscopes to the lab for this service.

SPECIMEN COLLECTION AND HANDLING:

Note: Collect and handle all potentially infectious specimens by following Standard Precautions.

Specimens:

- Vaginal discharge
- Urethral discharge
- Urethral-mucosa scrapings

1.0 Place approximately 0.5 mL of 0.85% non-bacteriostatic normal saline in a small test tube (10 x 75 mm). The saline must be at room temperature.

2.0 Collect vaginal material on a swab by rubbing the vaginal wall or by collecting material from the posterior fornix and emulsify in saline.

3.0 Label the tube with a patient ID label.

4.0 The sample should be maintained at room temperature (do not refrigerate) and examine within 15 minutes of collection.

TESTING PROCEDURE:

1.0 Gently mix the specimen and place one drop of specimen on each of two (2) slides, using a transfer pipet.

2.0 Place a cover slip on one (1) slide, being careful to avoid trapping air bubbles. Add one (1) drop of KOH reagent to the second slide as follows:

a. Hold the reagent dropper upright and point away from yourself. Grasp the middle with the thumb and forefinger and squeeze gently to break the ampoule inside the dropper.
Caution: Break ampoule close to the center one time only. Do not manipulate dropper any further as the plastic may puncture and injury may occur.

b. Tap the bottom of the dropper on table top a few times then invert for convenient drop-by-drop dispensing of the reagent. Add one (1) drop to the second slide.

c. Place a cover slip over the sample and tap down gently.

3.0 Adjust the microscope to obtain optimum contrast.

4.0 **SALINE PREP:** Scan the entire slide, using **10x** (low power field – **lpf**) and **40x** (high power field – **hpf**) objectives for the following:

- Budding yeast
- Pseudohyphae (fungal elements)
- Motile or non-motile Trichomonas
- White blood cells (WBC) > 10 cells / high power field (hpf)
- Clue Cells

5.0 **KOH PREP:** examining the KOH slide prep after the saline prep allows the KOH time to clear the cellular debris, thus making the observation of pseudophyphae easier.

6.0 The examination should be conducted within 5 minutes of the addition of the KOH to prevent the formation of drying artifacts.

7.0 In most cases, the presence of pseudohyphae and budding yeast can be determined using low power (10x). The KOH prep is not suitable for the examination of the other agent associated with vaginitis.

8.0 Record results in patients' chart for documentation.

9.0 Discard slides into a puncture proof container.

REPORTING RESULTS:

1.0 **Negative:** Indicates the absence of the cellular elements being evaluated.

2.0 **Positive:** Indicates the presence of any of the following cellular elements:

- ***Trichomonas vaginalis***
  Trichomonas are flagellates that have 3-5 anterior and 1 posterior flagella. They have rapid jerky motility that can be seen under low power. However, their numbers vary in clinical specimens and scanning the whole slide is essential. Also, Trichomonas has limited survival outside the host. Therefore, examining the specimen within 15 minutes is critical. As viability decreases, they become spherical and non-motile. Examination under high power may still identify spherical cells with flagellar movement.

- ***Budding yeast or psuedohyphae***
  Candida albicans is the most frequently encountered Candida sp. associated with vaginal infections, but the organism does occur in low numbers as part of the normal vaginal flora. There are two morphologic forms that are seen in clinical specimens, the yeast phase and pseudohyphae. The yeast phase is about the sizes of a red blood cell (7 – 8 micron, pear shaped) and can be seen as a single cell with or without a bud. Psuedohyphae appear as thick walled tube-like structures. **Note:** Low numbers of yeast, in the absence of clinical signs and symptoms, are considered part of the normal vaginal flora.

- ***> 10 White blood cells per high power field (WBC/hpf)***
- **> 20% Clue Cells**

3.0 Results may be manually written in the patient's chart and must include the date/ time of test and the initials of the person who performed the test. Positive test results must include the number of cellular elements seen per **hpf** or **lpf**.

QUALITY CONTROL:

1.0 Commercial controls are not currently available for wet mount preparation. Review of the same specimen by multiple examiners can help determine accuracy of test results.

2.0 Examine KOH reagent for signs of deterioration (precipitated KOH crystals on the wall of the ampoule or floating in the reagent). Refer to Attachment B: 10% Potassium Hydroxide Reagent Droppers Package Insert

LIMITATIONS OF THE METHOD:

1.0 False negative wet prep exams, with or without the presence of white blood cells (WBCs), may be as high as 60% when compared to other laboratory procedures, such as culture.

2.0 There are several reasons for this lack of sensitivity:

- Extent of experience or lack of experience of the examiner
- Patient hygiene practices such as douching and intra-vaginal medication use
- And the limits of detection of the direct examination procedure
- The presence of >10 WBCs / hpf may result from a non-infectious process.

PROFICIENCY TESTING PROCEDURE:

1.0 Proficiency testing is provided by the University of Washington through an on-line PPM competency challenge program, approved by CAP, the College of American Pathologists.

2.0 Physicians and medical residents who perform this test participate in this program, for initial training competency assessment.

3.0 Medical residents may perform this competency on an annual basis.

4.0 The passing grade for this on-line program is 80%.

5.0 Incorrect results will be investigated and retraining performed if necessary.

INFECTION CONTROL:

Handle and dispose of all materials coming in contact with blood according to universal precautions. Any spills or leakage must be cleaned with a disinfectant (10% bleach).

SAFETY:

Sharps precautions must be utilized or followed at all times.

REFERENCES:

1. NCCLS. *Provider-Performed Microscopy Testing; Approved Guideline*. NCCLS document HS2-A. NCCLS, Wayne, PA, 2003.

2. Henry, J B: Clinical Diagnosis and Management by Laboratory Methods, W.B. Saunders Company, Philadelphia, 2001.

ORIGINAL DATE:
July 2006

INITIATED BY:
Nellie Gillman, POCC

REVISED:
June 2011
February 2012