JOHN H. STROGER HOSPITAL of COOK COUNTY
DEPARTMENT of PATHOLOGY

POINT OF CARE TESTING

HEALTHCARE PROVIDER TRAINING

PROVIDER (Physician)
PERFORMED MICROSCOPY
(PPM)
Detection of causes of vaginitis and vaginal discharge by direct microscopy of wet mount and KOH slide smears at the point of care by trained healthcare professionals.

Determination of the pH of vaginal secretions as an adjunct in the diagnosis of bacterial vaginosis.
PURPOSE

To determine the presence or absence of Trichomonas, Candida, Clue Cells and Amniotic fluid in vaginal secretions.
INTENDED USE

• Only authorized trained healthcare professionals should perform this test (Physicians, Physician Assistants, and Advanced Practice Nurses).
PRINCIPLE

A wet preparation of vaginal fluid is evaluated microscopically for characteristic features.

The specimen’s pH, odor and the nature of the discharge are also considered in the evaluation process.

A Saline wet mount identifies clue cells, yeast/fungi and trichomonas.

A KOH mount will destroy all other cells except yeast and fungi.

A slide smear identifies ferning.
YEAST / FUNGI (CANDIDA)

- Candida albicans is a saprophytic yeast that exists as part of the endogenous flora of the vagina which sometimes become an opportunistic pathogen especially in the immunocompromised host or when a woman has taken antibiotics. Candida albicans is the second most common cause of vaginitis.

- The clinical manifestations include pruritis, burning, dysuria, and dyspareunia. Excoriation with secondary infection may occur. The vaginal discharge is frequently thick, white, and curd or cottage cheese-like.

- Yeast cells are larger than bacteria and smaller than vaginal epithelial cells. They often approximate the size of red blood cells. Unlike biconcave red blood cells, yeasts are spherical to ovoid. Yeasts have thick refractile cell walls. When present, small budding daughter cells clearly distinguish yeasts. Yeast cells are not destroyed by 10% KOH unlike all human cells.
TRICHOMONAS

- *Trichomonas vaginalis* is primarily a cause of vaginal infection but is also capable of infecting the urethra, periurethral glands, bladder and prostate. Its normal habitat is the vagina in women and the prostate in men.

- *T. vaginalis* is a pear-shaped single celled protozoan with a single nucleus. An additional important morphologic feature is an undulating membrane that originates anteriorly and extends posteriorly into a long flagellum. Several smaller flagella are present as well, but usually not seen well in unstained preparations.

- Trichomonas vaginitis is characterized by a pruritis, a thin grey or greenish discharge with an unpleasant odor and sometimes dysuria. A wet mount demonstrates the small organism moving chaotically with a rapid, jerky, rotating, nondirectional leaf-like motion. Rippling of the undulating membrane may be seen for several hours after cessation of organism motility.
BACTERIAL VAGINOSIS (BV)

• Clue Cells are epithelial cells of vaginal origin that are covered with bacteria. Clue cells have shaggy or beaded cell borders due to the adherence of coccobacilli. Most of the cell surface should be covered by bacteria for it to be identified as a clue cell. The presence of occasional irregular granules in the cytoplasm of squamous cells should be distinguished from adherant bacteria.

• Nitrazine paper can be used to test the pH of vaginal secretions. A pH greater than 4.5 is supportive of the diagnosis of BV.

• The discharge may have an amine or “fishy” odor which is accentuated by the addition of 10% KOH solution. This is the so-called “whiff test”.

• BV has been associated with an increased risk of pre-mature labor.
SPECIMEN COLLECTION

Vaginal secretions are collected from the posterior vaginal pool obtained by a speculum that has not been lubricated with petroleum jelly (water based Lubricants are O.K. – “Surgilube, and K-Y”).

Saturate a cotton tipped swab with the Specimen.
REAGENT

1. 10% Potassium hydroxide (KOH)
2. 0.85% Sodium chloride (SALINE)

• Make sure the opened dates and the expiration dates are written on the reagent vials.

• KOH reagent can be obtained from the Microbiology Laboratory staff, Pathology Dept. while SALINE is stored in the main storeroom of the John Stroger Hospital.
PROCEDURE FOR KOH MOUNT

- Allow smeared glass slide to air dry for 5 to 7 minutes.
- Add 1 drop of KOH solution over smear.
- Drop a cover-slip over the smear.
- Examine slide with a phase contrast microscope.

NB: The fluid should completely fill the area under the cover-slip without over flowing the area or causing the cover-slip to float.
Canisida albicans on KOH prep
Candida albicans (PAS stain)*

Yeast cell

Branching pseudohyphae

*Provided by R. Scott Pore, M.D.
FERNING for Amniotic Fluid

- The Fern test when it is used in conjunction with Nitrazine pH paper is highly sensitive and specific for the detection of amniotic fluid (indicating ruptured membranes). The test may be positive as early as 12 weeks of gestation.

- The presence of amniotic fluid is revealed by an elaborate arborized crystallization pattern (Ferning) as the specimen dries.

- Common contaminants such as blood, urine, meconium (by itself indicates ruptured membranes) semen or alkaline antiseptic solutions may cause false negative results if present in high concentrations. A false negative may also occur if several hours have elapsed from the time the membrane ruptures to the time of testing.

- Inadvertent contamination of the specimen by cervical mucus may cause a false positive result but the arborization pattern is less elaborate and normally will not form after the first trimester of pregnancy.
FERN TEST

Procedure:
1. Vaginal smear on a clean glass slide is air dried for 5 to 7 minutes.
2. Under low power (10X lens) microscope examine slide for characteristic ferning pattern.

Rupture of the membranes is premature if it has preceded the onset of labor by any amount of time. An accurate diagnosis is critical although difficult to accomplish. However, it is essential because both mother and fetus may be endangered if the condition remains undiagnosed and untreated.
FERN TEST LIMITATIONS

- A false negative result may occur if several hours have elapsed from the time the membrane ruptures to the time of testing and by contamination with blood, urine or antiseptics.

- A false positive result can also be obtained.
WET MOUNT PROCEDURE

A cotton tipped swab with specimen is smeared on a clean glass slide and allow to air dry for KOH mount; the swab is then inserted into a sterile tube (red top tube) with 2 to 3 drops (less than $\frac{1}{2}$ ml) of saline solution.

1. Thoroughly mix cotton swab into the saline solution.
2. Place 2 or 3 drops of the mixed solution on a clean glass slide (this can be accomplished by allowing the swab to drip onto the slide).
3. Place a cover-slip over the slide preparation.
4. Examine the slide with a brightfield or phase microscope.
Trichomonas – wet mount
(WBC to left for size comparison)
Clue cell – unstained smear
Clue Cells – stained smear
PROCEDURE
NITRAZINE pH Paper (NP) TEST

1. Tear approximately 2 to 3 inch of NP
2. Either (a) dip the strip of NP into the speculum with the specimen or (b) saturate a cotton swab with the specimen and use it to moisten the NP strip.
3. Shake off any excess (fluid) specimen on NP strip onto gauze or paper towel.
4. Immediately place the NP strip against the NP color chart to compare the color change and read the matching pH color accordingly.
5. Document result in pH chart along with microscopic test result.

NOTE: YOU MUST ALSO TAKE THE NITRAZINE PAPER TRAINING TO PERFORM THIS TEST
• Record the result of the microscopic exam in the patient’s chart as follows:

  – **Fern Test**: negative or positive
  – **KOH Prep**: negative or positive for fungi
  – **Wet mount**: identify clue cells or organisms seen or negative if none seen.
UNIVERSAL PRECAUTIONS

• Always observe departmental universal precautions at all times.
  – Wear gloves and protective barriers as needed and if required.
  – Follow established protocols to properly dispose of contaminated wastes and materials.
MICROSCOPE MAINTENANCE

• After each use, the clinician should use lens paper to clean the lenses.
  – Clean any residue or immersion oil from the objectives with an alcohol prep pad. Do not use acetone or any other solvent.
  – Clean the eyepiece and objectives with lens paper or lint-free tissue. Do not use facial tissue or toilet paper.
  – Clean the arm, table and base with recommended alcohol wipes, or germicidal disinfectant.
  - Maintain the cleaning record at the unit with the initials of the person performing the cleaning.
NEXT STEP

• Take AND PASS the test. Your learning file on the CCH Intranet will have a record of your on-line training permanently. This can be accessed by your Department as proof of training.

• Thank you.