**LOURDES HOSPITAL**
**169 Riverside Drive**
**Binghamton, New York 13905**
**LAB MANUAL**

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**SUBJECT: Provider Performed Wet Prep/KOH** **ORIGIN DATE: 04/95**

**REVIEWED: 6/20/2016** **REVISED: 05/02**
**REGULATORY REFERENCES:** **CROSS REFERENCES:**
**NYS, CAP, JCAHO**

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**POLICY** Examination of a wet preparation of vaginal and urethral discharge is the most useful rapid diagnostic test for such specimens. Motile trophozoites of *Trichomonas* can be visualized in 80% of the cases, and budding cells and pseudo hyphae can also be identified.

Microscope slides made from patient specimens can be examined by the provider, M.D., P.A.,FNP, under low and high power for the presence of actively moving organisms.
The provider will participate in proficiency testing provided by the lab.

**PROCEDURE:**

SPECIMEN TYPE

a. vaginal discharge
b. urethral discharge
c. penile discharge
d. Skin, hair, or nails

COLLECTION- Female: The Health Care Provider (HCP) collects a specimen from vagina or urethra on a sterile swab. Patient should not have douched for 3 to 4 days prior to specimen collection.
Male: The HCP collects urethral discharge or prostatic secretions on a sterile swab.

The swab is placed in a test tube with approximately 1 ml of sterile saline. Excess saline may impair interpretation of the test.

Storage**:** The specimen must be read by the HCP within one hour of collection. Do not refrigerate, as refrigerator temperatures inhibit motility and have a deleterious effect on these organisms. Specimens must be rejected and not read if more than 1 hour old.

Specimens can be sent to the Laboratory to be read if submitted in Amies transport media. These specimens are acceptable for up to 24 hours. Specimen must be logged on the transfer log.

See procedure section for collection of hair, skin and nails.

REAGENTS AND MATERIALS

1. 0.85 % NaCl – Prepared commercially in 1 liter sterile plastic bottles. Store at room temperature. Label bottles with an expiration date of 6 months following opening, or the expiration date specified by the manufacturer, which ever is sooner.

2. 10% Potassium hydroxide (KOH) solution—Take 10g potassium hydroxide and dissolve in 90ml of distilled water. Stable for 1 month. Alternatively, a commercially prepared 10% KOH solution can be used. Store at room temperature until the expiration date.
3. 10% KOH plus Shaeffler's ink solution—8ml prepared 10% KOH and 2ml Shaeffer's permanent black ink. Store at room temperature. Stable for 6 months.
4. For collection: scraping implement such as a sterile blade or skin and nail, forceps for hair, sterile swabs for vaginal secretions. No patient preparation is needed.
5. 70% Alcohol (alcohol pads)
6. Disposable glass or plastic pipettes
7. Glass slides
8. Coverslips
9. Small test tube

IDENTIFICATION and LABELING: the patient will be identified using 2 identifiers, patient name and date-of-birth. The specimen will be labeled with the 2 identifiers.

TEST PERFORMANCE

1. Collect the specimen as follows:
a) Skin: Cleanse the skin with 70% alcohol to remove any contamination. If a characteristic dermatophyte "ring" is present on the skin, collect samples from the outer margins of the ring at its junction with the normal skin. Otherwise, collect samples from areas where the skin appears to be scaling. Use the edge of a glass slide or scalpel to scrape off the surface skin cells. Collect scales on a slide or sterile cup.
b) Nail: Clean the nail with 70% alcohol to remove contamination. Scrape away top layers of the nail and collect subsurface material. Collect material onto a slide or sterile cup.
c) Hair: Extract a few hairs with forceps from the affected area. Collect into a sterile cup.
d) Using a culturette swab with gel preservative (for example, Amies gel swab), collect vaginal material and return swab to preservative sheath.

2. Apply the patient's specimen to a small area of a clean microscope slide.
3. Add 1 drop of 10% KOH solution and mix.
4. Cover the specimen with a coverslip
5. Allow the prep to incubate at room temperature until the material has been celared. This generally takes 5-15 minutes. The slide may be gently warmed under a flame to speed up the clearing process. DO NOT allow specimen to boil.
6. Examine the wet mount with the low-power (10X) objective and low light.
a) Examine the entire cover-slipped area for motile flagellates, budding yeast cells, pseudohyphae, and clue cells.
b) Prepare a second slide, adding 1 drop of KOH. The addition of 10% KOH to the preparation may elicit a "fishy", amine-like odor associated with bacterial vaginosis. This is called the "Whiff test".

Limitations:

a. It is very important that specimens be examined within 1 hour of collection. After 1 hour, the organisms may round up, lose their motility and eventually die.
b. Wet mounts have failed to detect the presence of *T. vaginalis* in 10-25% of infected patients

## RESULT REPORTING

The patient's results must be recorded in the patients chart as well as the accession log with patient's name, date of birth, the date and time of the test, and signature of the person performing the test

If motile flagellates (axostyle and undulating membrane) are seen, report the presence of *Trichomonas vaginalis.*

If epithelial cells which are completely covered with tiny coccobacilli are noted, report the presence of Clue cells.

Report the presence of budding yeast cells, pseudohyphae, red blood cells and white blood cells. If a "fishy" odor is noted during the Whiff test, report a positive whiff test.

## QUALITY CONTROL

a. Check direct mount reagents each time they are used. The saline should be clear, with no visible contamination. Replace reagent if cloudiness is noted.
b. The microscope should have been calibrated within the last 12 months and the objectives and oculars used in the calibration procedure should be used for all measurements on the microscope.
c. Due to the nature and the fragility of the bacteria, there is no external quality control for Wet Prep procedures.

## FOLLOW UP RECOMMENDATIONS

Alternate diagnostic methods include culture and monoclonal antigen detection kits. These procedures are available only through a reference laboratory.

**Next Review:**

**AUTHORIZATIONS:**

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| _____ | _____ |
| Medical Director | **Date** |
| _____ | _____ |
| Clinical Manager | **Date** |