

Guthrie Medical Group
POLICY & PROCEDURE

SECTION: Department	SUBJECT: PPM Procedure - Wet Mount and KOH	DEPT. Point-of-Care Testing Regional Labs
EFFECTIVE: 7/27/2016		POLICY# GMG-700-2019
SUPERCEDES: 3/04		PAGE # 1 of 6
DISTRIBUTION: PPM permitted offices		

I. INTENDED USE:

A wet mount is a microscopic procedure performed to examine material collected from a specimen suspended in a drop of liquid on a glass slide. A wet mount is used to view cells and organisms for motility, morphological characteristics and identification. Specifically, it is used for the presence or absence of bacteria (clue cells), Trichomonas (motile flagellate) and human cellular elements.

Direct microscopic examination of vaginal secretions is instrumental in the diagnosis of Candidiasis, bacterial vaginosis caused by Gardnerella vaginalis and infections caused by the parasite, Trichomonas vaginalis. Potassium hydroxide, when added to a slide, dissolves many interfering structures so that yeast cells can easily be seen and identified.

II. EQUIPMENT, REAGENTS and SUPPLIES

Product	Epro #	Vendor	Manufacturer
Physiologic Saline (NaCl 0.9%), sterile	71962	Cardinal Health	Cardinal
Glass Slides, Frosted	11611	Cardinal Health	Cardinal
Glass Coverslip (glasses) 22x22	12236	Cardinal Health	Cardinal
KOH 10% Dropper	19780	Cardinal Health	Remel
Transfer Pipets	2221	Cardinal Health	Cardinal
Sterile Swabs, Culture Swab	1395	Cardinal Health	Cardinal
Gloves			
Sharps Container			
Microscope	Contact the Regional Office Lab Coordinator		

III. QUALITY CONTROL:

External quality control materials are not available. Staff competency assessment and proficiency will be used to verify reliability of patient test results.

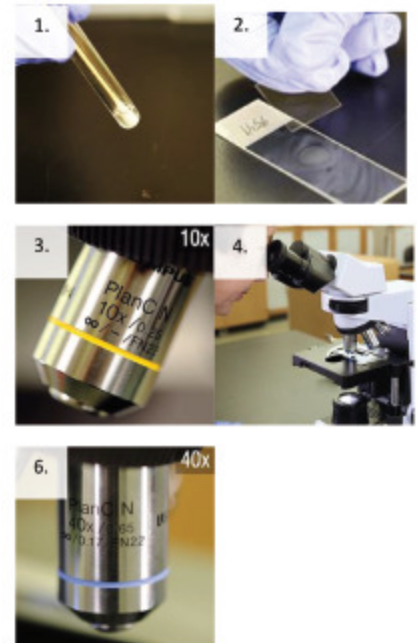
IV. SPECIMEN COLLECTION

A wet mount is a microscopic procedure performed to examine material collected from a specimen suspended in a drop of liquid on a glass slide. A wet mount is used to view cells and organisms for motility, morphological characteristics and identification. Specifically, it is used for the presence or absence of bacteria, fungi, parasites, and human cellular elements.

1. Prior to collecting specimens, perform hand hygiene and put on gloves. Standard precautions should be taken when collecting and handling blood or body fluid specimens.
2. Using sterile cotton swabs, collect two swabs of vaginal discharge from mucosa high in the vaginal canal obtained during an examination using a speculum.
3. Appropriately label the specimen.

V. WET MOUNT PROCEDURE

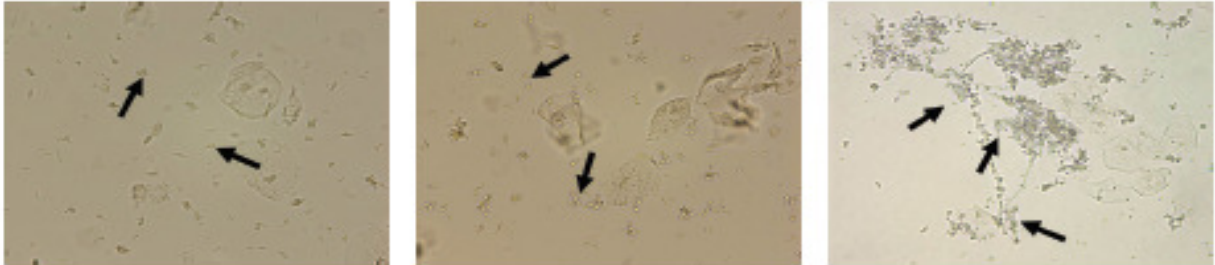
1. Place one swab in a clean test tube containing 0.5 ml of normal (0.9%) saline. Ensure that the specimen tube has at least 0.5 ml of saline and no more than 1.0 ml.
2. Gently twist the swabs in saline to dislodge particles from the swab tip.
3. Reseal the tube to preserve the specimen.
4. Do not refrigerate the specimens.
5. Perform testing immediately
6. Using a sterile transfer pipette or dropper, gently mix the specimen and remove some of the specimen from the tube and place one drop on a clean, microscope slide labeled with the patient's name and the word "saline"
7. Immediately put a coverslip over the specimen for examination. A microscope review of the slide should be performed as soon as possible to confirm the presence or absence of Trichomonas.
8. Place slide on microscope for examination and focus using low power (10X) and low light.
9. Scan the entire slide reading at least 10 fields.
10. Identify objects using high power (40X).
11. Record findings as the presence or absence of clue cells/hyphae or yeast / trichomonads.
12. Dispose of the slide into an appropriate sharps container.



COMMON WET MOUNT MICROSCOPIC FINDINGS:

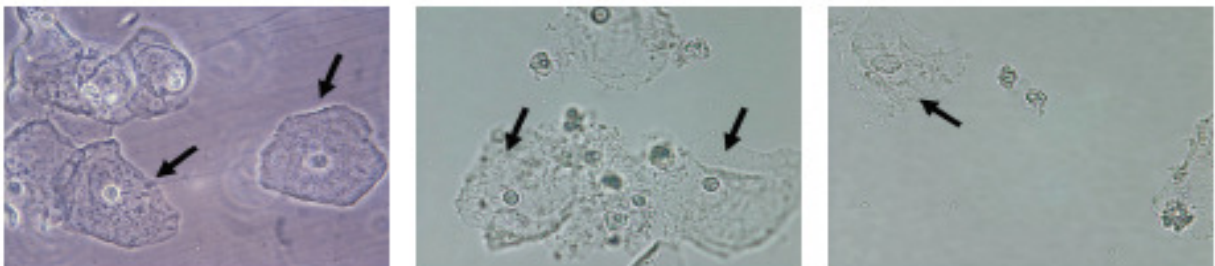
Yeast

Yeast are unicellular fungi that appear commonly in vaginal discharge wet mounts. Yeast can appear as budding yeast or yeast with pseudohyphae, an elongated filament-like string of attached cells. In the images below, the arrows indicate examples of yeast.



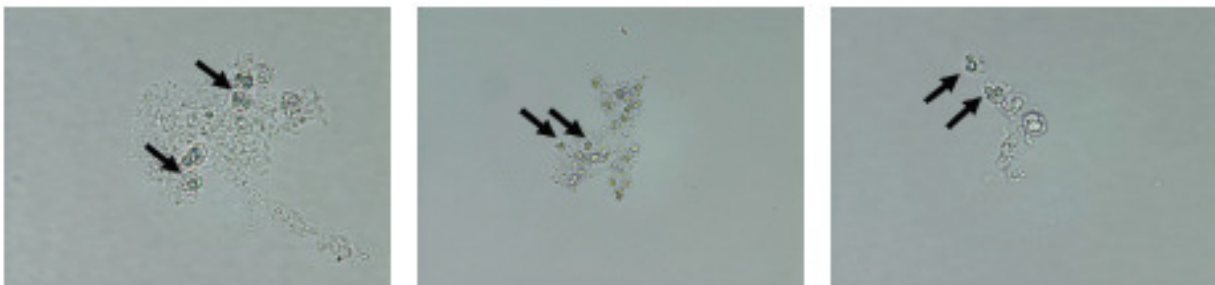
Squamous Epithelial Cells

The majority of cells observed in a normal vaginal wet prep will be vaginal epithelial cells. Squamous epithelial cells are large and flat with an irregular shape, distinct borders, and a single nucleus. In the images below, the arrows indicate examples of squamous epithelial cells.



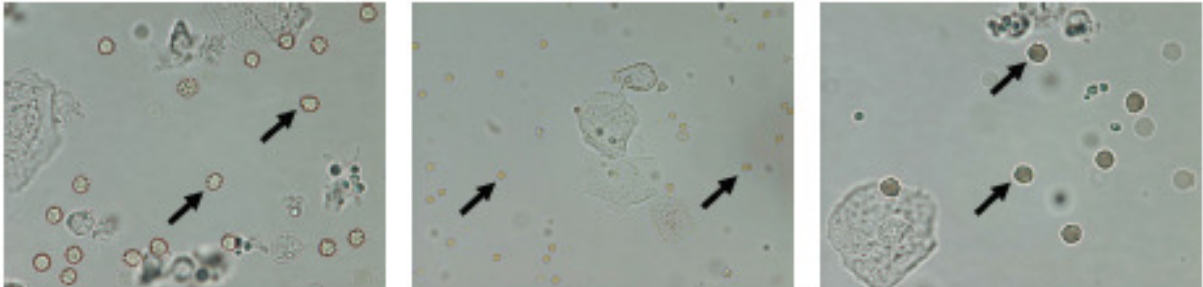
White Blood Cells

White blood cells (WBCs) are a normal component of vaginal flora. WBCs are small and have a multi-lobed nucleus and appear dark and granular. WBCs can be elevated in infections involving *Chlamydia*, *Trichomonas vaginitis*, herpes, and *Neisseria gonorrhoeae*. A ratio of one WBC for every epithelial cell is considered within normal limits. In the images below, the arrows indicate examples of white blood cells.



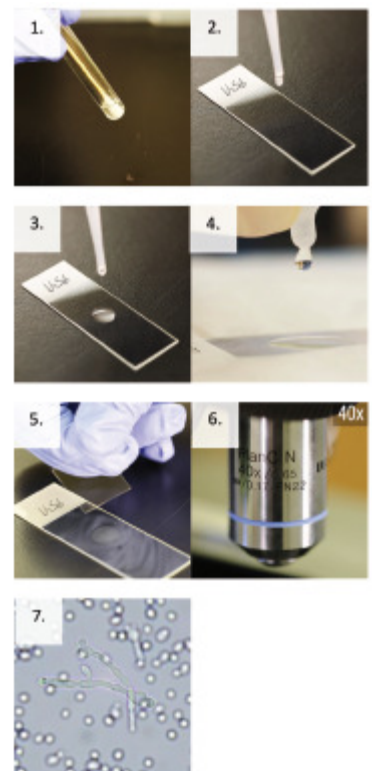
Red Blood Cells

The presence of red blood cells may indicate bleeding during the collection process. In the images below, the arrows indicate examples of red blood cells.



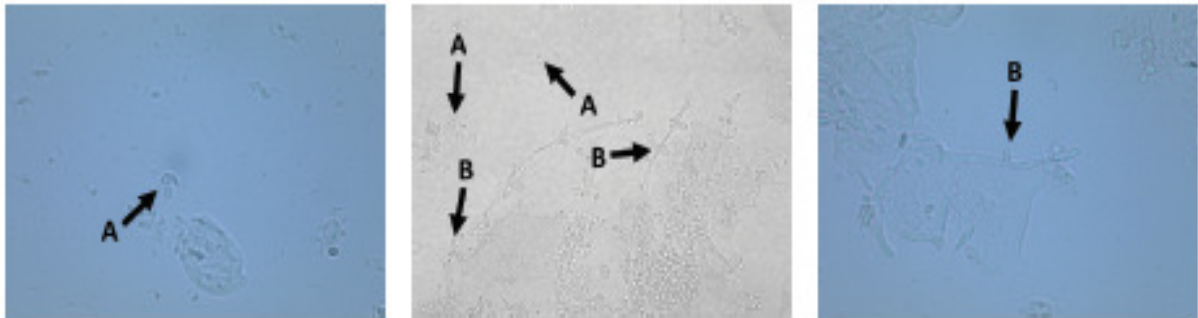
VI. KOH PROCEDURE

1. Collect vaginal specimen as described in specimen collection above.
2. Place a swab in a clean test tube containing 0.5 ml of normal (0.9%) saline. Ensure that the specimen tube has at least 0.5 ml of saline and no more than 1.0 ml.
3. Gently twist the swabs in saline to dislodge particles from the swab tip.
4. Reseal the tube to preserve the specimen.
5. Do not refrigerate the specimens.
6. Perform testing immediately
7. Using a sterile transfer pipette or dropper, remove one drop of the specimen from the tube.
8. Place one drop on a clean microscope slide with the patient's name and the word "KOH".
9. Check the slide for a "fishy", amine odor and note presence or absence. The odor indicates anaerobic bacteria overgrowth.
10. Without touching the specimen with the dropper tip, add one drop of 10% Potassium hydroxide (KOH) directly to the drop of specimen on the slide.
11. Wait up to 5 minutes to allow cellular tissue and debris to dissolve. Place a coverslip over the slide.
12. Focus the slide using low power (10X). Scan at least 10 fields using high dry power (40X).
13. Examine for budding yeast or yeast with pseudohyphae.
14. Record results as the presence or absence of yeast or hyphae.



COMMON KOH PREPARATION MICROSCOPIC FINDINGS

Yeasts are unicellular fungi that appear commonly in vaginal discharge wet mounts. Yeasts are larger than bacteria, approximately the size and shape of the nuclei of epithelial cells. Individual cells of yeast propagate by budding out similar cells from their surface. In PPM observations, yeasts are indicated by a loose arrangement of budding cells. In many fungi, the budding cells remain attached to the parent cell resulting in an elongated filament-like string of attached cells, pseudohyphae, with a cell budding off the tubular structure. In the images below, the arrows indicated by "A" are examples of yeast and the arrows indicated by "B" are examples of pseudohyphae.



VII. INTERPRETATION

The presence of *Trichomonas*, clue cells or *Candida* species is instrumental in the diagnosis of infection but must be consistent with the clinical signs and symptoms presented by the patient. The following is a listing of additional information which supports each diagnosis.

1. **Candidiasis** – *Candida albicans* can be found in low numbers in the vagina, anterior urethra and external genitalia of healthy individuals. When the quantity increases and is accompanied by a thick, curd-like discharge, the diagnosis of *Candida* Vaginitis is more likely. *Candida* vaginitis very often is found in conjunction with other conditions such as diabetes mellitus, pregnancy and prolonged antibiotic therapy.
2. ***Trichomonas*** – classically, the discharge produced with this condition is copious, frothy yellow or yellow-green and collects in the vaginal fornix. In women, the organism feeds on the mucosal surface of the vagina, ingesting bacteria and leukocytes. It is also capable of infecting the urethra, periurethral glands, bladder and prostate.
3. ***Gardnerella vaginalis*** is associated with bacterial vaginosis (nonspecific vaginitis). Bacterial vaginosis is a defined syndrome, the hallmark of which is an excessive, malodorous vaginal discharge, associated with a significant increase in the number of *Gardnerella* organisms. It is theorized that the *Gardnerella* work synergistically with anaerobic bacteria belonging to the *Bacteroides* and *Peptococcus* species. A minimum diagnostic requirement for bacterial vaginosis is the presence of the following:
 - a. Excessive vaginal discharge
 - b. Vaginal pH of >4.5
 - c. Presence of "clue cells"
 - d. A "fishy," amine odor when KOH is added to the secretions on a slide.** *Gardnerella* organisms should not be confused with lactobacilli, which are considered to be normal vaginal flora.

VIII. RESULTS REPORTING

Enter results in the patient electronic medical record using the enter / edit activity in Epic. Refer to policy GMG-700-2030.

1. Note the presence of one or more of the following:
 - a. Budding yeast, presumptive of the Candida species.
 - b. Trichomonas vaginalis
 - c. Gardnerella vaginalis
2. Note the quantity of any of the above organism as either:
1+, 2+, 3+, 4+ or Rare, Occasional, Frequent, Numerous
3. Note the appearance of the secretions or discharge examined.

Normal Values:

Candida – no or rare budding yeast, no discharge.

Trichomonas – no trichomonas

Gardnerella – rare to occasional tiny coccobacilli, with frequent to numerous Lactobacilli, no clue cells.

IX. REFERENCES:

1. CLSI Physician and Nonphysician Provider-Performed Microscopy Testing: Approved Guideline-Second Edition POCT 10-A2 ; 2011
2. CMS Provider Performed Microscopy Procedures, Wet Mount and KOH, Feb. 2016
3. Bauer, John M.D. Clinical Laboratory Methods, 8th ed. C.V. Mosby Company, St. Louis, 1974: 50-60.
4. Todd, Sanford and Davidsohn, Clinical Diagnosis and Management by Laboratory Methods, 16th ed. W.B. Saunders Co. Philadelphia, 1985: 559-629
5. OnFocus, internet site, accessed 8/4/2014 <http://onfocus-laboratories.software.informer.com/>

Procedure written by: Sabena Lukasavage, BS
Regional Laboratory Coordinator
Guthrie Medical Group Laboratories
Date: 3/3/2004

Procedure Revised by Tonya Wilhelm. MT 7/26/2016

Key Contact: POCT Coordinator, Laboratory Medical Director

Reviewed and Approved by: _____
Laboratory Medical Director* Date _____

Reviewed and Approved by: _____
Laboratory Medical Director* Date _____

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Laboratory Medical Director* Date _____

Reviewed and Approved by: _____
Laboratory Medical Director* Date _____

*Original signed document is available in the Regional Laboratory Coordinator’s Office