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**48667.258 Saline and KOH Vaginal Wet Mounts**

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**Approval and Periodic Review Signatures**

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Periodic review	Designated Reviewer	3/22/2021	3.1	<i>Barbara Haller, MD, PhD</i> Barbara Haller	
Approval	Lab Director	9/27/2019	3.0	<i>Barbara Haller, MD, PhD</i> Barbara Haller	
Approval	Laboratory Administrator	9/26/2019	3.0	<i>Mary Eugenio-Allen</i> Mary Eugenio-Allen	

Signatures from prior revisions are not listed.

**Version History**

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# SALINE and KOH VAGINAL WET MOUNTS

## PURPOSE

Vaginal fluid analysis is used for the identification of motile **Trichomonad organisms,"clue cells"** associated with bacterial vaginosis (BV) and hyphae or budding yeasts associated with vaginal candidiasis. *Trichomonas vaginalis* is a pathogenic flagellate that infects the urogenital tracts of males and females. The infection is primarily a sexually transmitted infection and is thought to be the most common curable sexually transmitted infection among sexually active women. Leukocytes and **budding yeasts** or **hyphae** can also be detected by saline wet mount and are a cause of vulvovaginal candidiasis. Addition of KOH hydrolyzes cellular material aiding in observation of yeasts and pseudohyphae.

## PRINCIPLE

A vaginal wet mount preparation is a test where a sample of vaginal discharge is observed by wet mount microscopy. The swab collected sample is placed on a clean glass slide and mixed with either 0.9% saline or 10% potassium hydroxide (KOH). Saline preserves cell membranes and allows observation of motile trichomonads, and vaginal epithelial cells coated with bacteria (clue cells), and white blood cells (WBCs). If a yeast infection is suspected, the addition of 10% KOH will hydrolyze trichomonads, cells, and bacteria to provide a clearer view of yeasts and pseudohyphae. In many cases of BV or trichomoniasis infection, addition of KOH to the vaginal discharge will produce a strong amine or fishy odor.

## TESTING PERSONNEL

- Qualified physicians, nurse practitioners, physician assistants, and midwives may qualify to enroll in the PPMP program. NOTE: residents and fellows with 2 years or more of residency training and a current California medical license may qualify to enroll in the PPMP program.
- Interns, residents and fellows enrolled in an ACGME approved training program may perform the test when supervised by a qualified, licensed provider.
- Testing personnel are required to take the initial training and competency assessment. Competency assessment (using 6 methods) must be completed after initial training and before technical duties are performed, six months after completion of initial training and annually thereafter.

## SPECIMEN

- Freshly collected vaginal wall swabs in a test tube with 0.5 mL saline
- Specimen labeling is not required when testing is performed in the presence of the patient and only the sample from one patient is tested at a time. If there is the potential for specimen mix-up, the sample tube must be labeled with patient’s full name and medical record number.

## EQUIPMENT, REAGENTS AND SUPPLIES

### A. Equipment:

Binocular microscope with 10x and 40x objectives. Preventive maintenance should be done at least annually by Biomed Services, evidenced by a dated label on the microscope.

### B. Reagents:

0.9% NaCl in a dropper bottle, available from SFGH Pharmacy.  
10% Potassium hydroxide (KOH) in a dropper bottle, available from SFGH Pharmacy.

### C. Supplies:

1. Disposable test tube.
2. Cotton swabs
3. Glass slides
4. Coverslips

## QAULTY CONTROL CHECK

- A. On each day of microscope use, perform a microscope check or check a positive QC slide.
- B. An acceptable microscope check is when the image is clear and the view is free of debris caused by dirty lenses. If the lenses are dirty, clean them with lens paper and lens cleaner.
- C. Preventive cleaning and maintenance of the microscope is performed annually by Biomed Services.

## PROCEDURE

- A. Using two patient identifiers (full name and date of birth or medical record number) verify patient identity and explain procedure to the patient and/or family.
- B. Observe universal precautions; wear gloves and other personal protective equipment as appropriate.
- C. Check the appearance of the 0.9% NaCl and 10% Potassium hydroxide (KOH) solutions for clarity and no visible contamination.

- If the solutions are clear, proceed with test.
- If the solutions are not clear, discard and obtain new solutions from the ZSFG Pharmacy, then proceed with test.

- D. Store the 0.9% NaCl and 10% KOH solutions at room temperature. Check the expiration dates. Only use the solutions if before the expiration date.
- E. Use warm water to lubricate the vaginal speculum. Lubricant can interfere with slide analysis. Use two cotton swabs together and swab the middle third of the lateral walls of the vagina. To prevent drying of the sample, place the swabs immediately in a test tube with 0.5 mL of normal saline. Hold specimen at room temperature.
- F. Gently mix the swabs in a test tube with 0.5 mL of saline. Apply a drop of the specimen-saline mixture on a clean slide.
- G. For KOH wet preparations, Apply 1 drop of 10% KOH to the vaginal secretion-saline mixture on the slide. A fishy odor suggests bacterial vaginosis.
- H. Cover the specimen with a coverslip. For KOH wet preparations, let the specimen stand for 2 to 5 minutes to allow for the clearing process to occur.
- I. Examine the wet mount with a low-power (10x) objective and low light. Close down the aperture diaphragm on the condenser.
- J. Review the entire coverslip to get a general impression of the specimen. Look first at low power (10x objective) and then at high power (40x objective). Look for and evaluate the types and numbers of the following cells.
  1. Clue cells: Epithelial cells with many small coccobacilli coating to the cell surface causing irregular borders and blurred edges.
  2. Leukocytes (WBC's): The polymorphonuclear (PMN) cell is the most commonly seen WBC and has a multi-segmented nucleus and granules in the cytoplasm.

And the following organisms:

2. Trichomonads: Motile organisms which are about the size of WBC's or larger and pear, round or triangular shape.
3. Yeast: Pseudohyphae of and budding yeast cells can be seen on a saline and KOH wet mounts.

- K. Dispose of swabs and other contaminated supplies in a Biohazard waste container. Slides should be discarded in sharps container.

## RESULTS AND PRECAUTIONS

- A. Motile flagellates for trichomonads must be seen to report a positive finding. Brownian motion (appearance of continuous "jiggling" of cells or organisms) is not true motility. True motility occurs when an organism shows directional movement and is moving by itself and not as part of the movement of the liquid toward the edges of the coverslip.
- B. A few WBC's (1-5 per high power field, HPF, i.e., visible field using the 40x objective) can be considered a normal finding. More than 5 WBC's / HPF often indicate vaginitis or cervicitis. WBC's with no obvious cause indicate the need for further workup for gonorrhea, Herpes simplex vaginitis, Chlamydia cervicitis, or atrophic vaginitis.
- C. Avoid confusing the following:
1. RBC's and yeast cells - look for buds or pseudohyphae. KOH solution should lyse RBC's as well.
  2. Pseudohyphae and cotton fibrils or epithelial cell borders. Confirm finding on KOH preparation. True pseudohyphae crisscross epidermal cells in a random fashion and the strands are usually of a uniform diameter.
  3. Non-motile organisms can be mistaken for WBC's or some types of Epithelial cells. Examine the wet mount within 15 minutes after collection to avoid non-motile organisms. There is usually an increase in WBC's.
  4. Coccobacilli and other bacteria attached to epithelial cells. "Clue cells" have ragged and obscure edges. Lactobacilli are reduced or absent. The presence of increased WBCs in the absence of clue cells, trichomonads, hyphae or budding yeast, consider other sources of infection or inflammation.

## REPORTING RESULTS

- A. Report the presence or absence of Trichomonas, yeast/pseudohyphae, and "clue cells." WBCs may be noted if present in significant numbers.
- B. Record results in the electronic medical record (EMR) or results sheet.

## REFERENCE VALUES:

Finding	Expected Result
Clue Cells	Absent
Trichomonads	Absent
Yeast and/or pseudohyphae	Absent
White Blood Cells	Few (0-5 / HPF)

## LIMITATIONS OF PROCEDURE

- A. The saline and KOH wet mount procedures should be done immediately for optimal recovery. A specimen left at room temperature or held at refrigerator temperature for more than an hour will result in problems such as rounded organisms, loss of motility of the trichomonad flagellate, or death of the organism.
- B. Saline wet mounts have been reported to detect *T. vaginalis* in only 75 to 85% of infected patients.
1. KOH preparation is more specific for the identification of yeast cells and pseudohyphae.
  2. Failure to visualize yeast and pseudohyphae on KOH wet mount does not completely exclude vaginal yeast or fungal infections.

## REFERENCES

- A. Kern, WE. 1985. Medical Mycology, a self-instructional text. F.A. Davis Company, Philadelphia, PA.
- B. Lowe, Shirley, and Saxe, Joanne. Microscope Procedures for Primary Care Providers. 1999. Lippincott Williams & Wilkins. 227 East Washington Square, Philadelphia PA 19106.
- C. NCCLS. Provider-Performed Microscopy Testing; Approved Guideline. NCCLS Document HS2-A, NCCLS, Wayne PA; 2003.

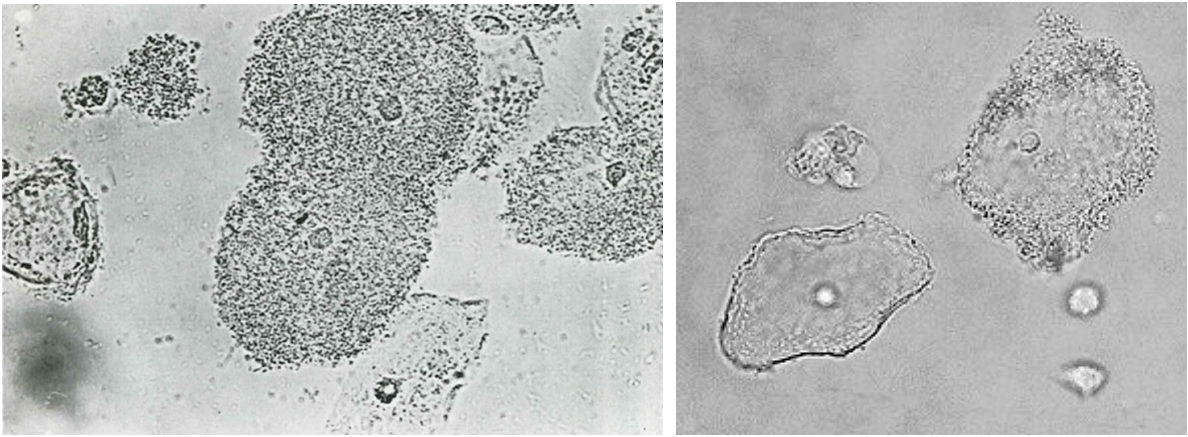
## DISTRIBUTION

- A. Point of Care Testing Master Manual
- B. Approved Point of Care Testing locations via POCT Website [www.SFGH-POCT.org](http://www.SFGH-POCT.org)

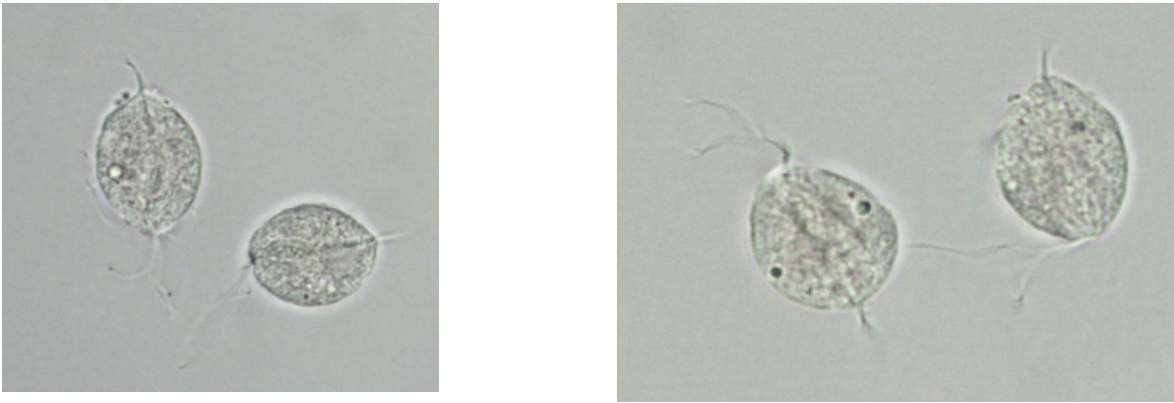
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**APPENDIX A: IMAGE REFERENCE EXAMPLES**

**“Clue Cells” (*Gardnerella vaginalis* – Bacterial Vaginosis)**



**Trichomonads (*Trichomonas Vaginalis*)**



**Yeast and/or Pseudohyphae (*Candida albicans*)**

