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48667.258 Saline and KOH Vaginal Wet Mounts

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Comments for version 1.0

Initial version
Removed: Smell the mixture. This is the "whiff" test for *Gardnerella vaginalis*. Reverted to edit from Dec 6 2017 11:10AMRemoved: Smell the mixture. This is the "whiff" test for *Gardnerella vaginalis*.
Removed "Draft" watermark
Added more images to Appendix
Removed mention of "Whiff test" in introduction
Added reference values

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	1/10/2018	1.0	Barbara Haller, MD, PhD Barbara Haller	
Approval	Laboratory Manager	1/03/2018	1.0	Mary Eugenio-Allen Mary Allen	
Approval	POCT Supervisor	12/12/2017	1.0	DEBORAH NEGRI	

Prior History

Date of Original: May 1, 1999

Date Revised: September 2009; Revised by: Marguerite Roemer, MT (ASCP) SM and Clayton Hooper, RN

Last Approved by: Barbara Haller, MD, PhD, Division Chief Microbiology and Eberhard Fiebig, MD, Director

12/2017 Update by C. Tolman-Salinas: Combined Saline and KOH wet mount procedures and updated reporting section to reflect steps for eCW reporting.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired

1.0	Approved and Current	Initial version	12/06/2017	1/12/2018	Indefinite

Author: Caroline Tolman-Salinas, CLS; Original Authors: Robyn Whidden, RN, NP and Mary Clancy, MS, MT
Approved by Barbara Haller on 1/10/2018.
Reviewed by Barbara Haller on 1/10/2018.

SALINE and KOH VAGINAL WET MOUNTS

PURPOSE

Vaginal fluid analysis is used for the identification of motile **Trichomonad organisms** and **"clue cells"** associated with bacterial vaginosis (BV). *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, 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 Licensed Physicians
- Qualified Licensed Nurse Practitioners, Physician Assistants, Midwives

Interns, residents and fellows enrolled in an ACGME approved training program may perform saline wet mount procedures when supervised by a qualified, licensed provider.

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 l0x and 40x objectives. Preventive maintenance should be done at least annually, 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

PROCEDURE

A. Using two patient identifiers 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 SFGH 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 (l0x) 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.

And the following organisms:

1. Coccobacilli: Many small bacilli are seen both on the epithelial cells and in the background.
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.

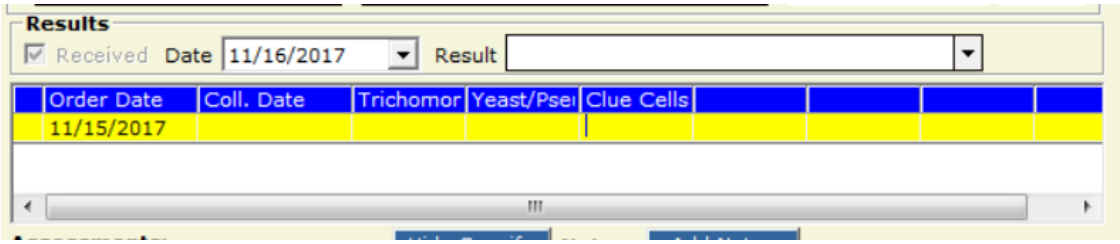
2. Pseudohyphae and cotton fibrils or epithelial cell borders. Confirm finding on KOH preparation. True pseudohyphae crisscross epidermal cells in a random fashion and the strands are usually of a uniform diameter.

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.

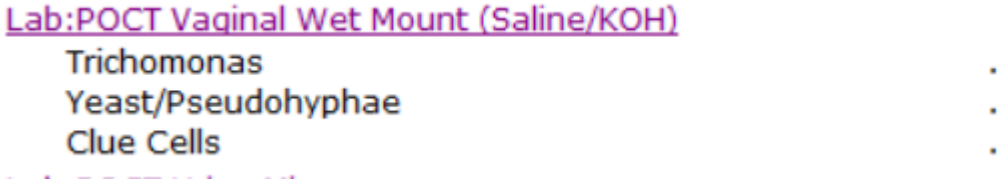
4. Coccobacilli and other bacteria attached to epithelial cells. "Clue cells" have ragged and obscure edges. Lactobacilli are reduced or absent. If there is an increase in WBC's with the "clue cells," a second infection needs to be considered.

REPORTING RESULTS

A. Order the test in eCW as "Lab: POCT Vaginal Wet Mount Saline/KOH":



B. Report the presence or absence of Trichomonas, yeast/pseudohyphae, and "clue cells."



REFERENCE VALUES:

Finding	Expected Result
Clue Cells	Absent
Trichomonads	Absent
Yeast and/or pseudohyphae	Absent

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.

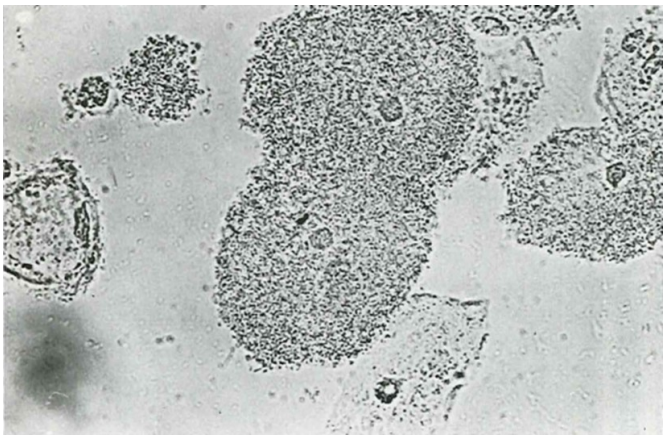
DISTRIBUTION

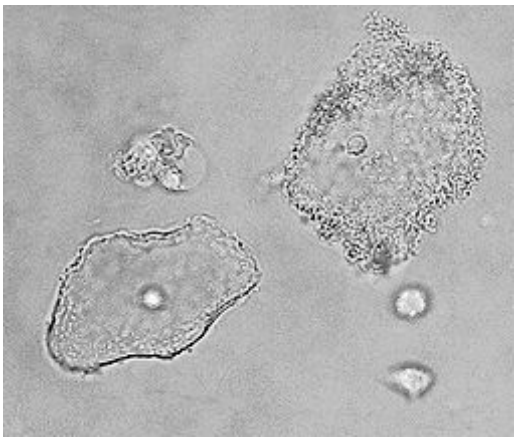
A. Point of Care Master Procedure Book (2M14).

B. Approved Point of Care Testing Locations.

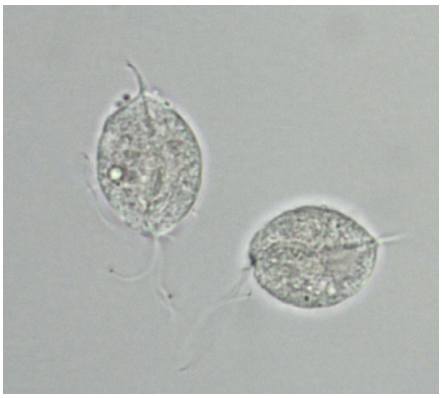
APPENDIX A: IMAGE REFERENCE EXAMPLES

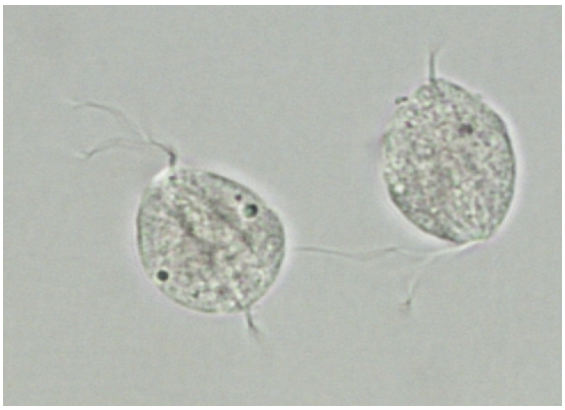
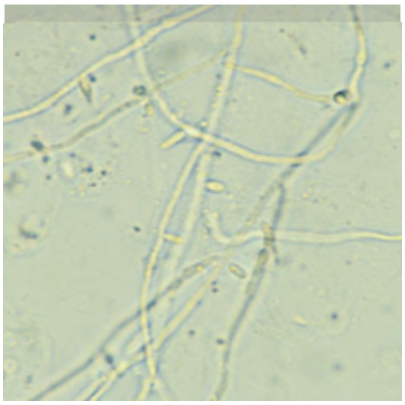
"Clue Cells" (*Gardnerella vaginalis* – Bacterial Vaginosis)





Trichomonads (*Trichomonas Vaginalis*)





Yeast and/or Pseudohyphae (*Candida albicans*)