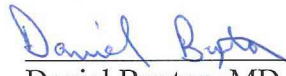


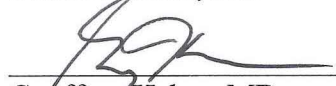


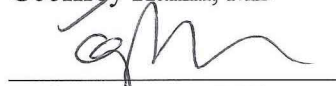
KHN Wet Prep Procedure

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Adopted: _____ Date: June 1, 2019

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I. PRINCIPLE

Trichomonas vaginalis infections are primarily diagnosed by detecting live motile flagellates from direct saline (wet) mounts. Microscope slides made from patient specimens can be examined under low and high power for the presence of actively moving organisms. Yeast cells and hyphae or pseudohyphae can also be detected on the saline wet mounts. Clue cells are vaginal epithelial cells that are covered with bacteria giving them a stippling appearance with fuzzy edges. They are a medical indication of bacterial vaginosis.

Trichomonas is associated with or may cause vaginitis. Diagnosis may be made by examination of vaginal (or urethral since males may carry the organism) exudate collected by the physician. The specimen should be examined immediately for the demonstration of actively motile flagellates. Direct microscopic examination of a wet preparation of vaginal discharge provides the simplest rapid diagnostic test for Trichomonas

Clue cells (indicative of G. vaginalis) are squamous epithelial cells covered with tiny bacilli, especially around the periphery, giving the cell a stippled appearance. Gardnerella vaginalis is a pleomorphic gram-negative to gram variable bacillus. G. vaginalis is found in the vagina of 25-45% of asymptomatic women. However, bacterial vaginosis is usually not present in the absence of the organism. Therefore, the role of G. vaginalis in bacterial vaginosis is uncertain. The diagnosis of bacterial vaginosis is more reliably made based on; vaginal secretion of pH greater than 5.0, a fishy amine odor upon addition of KOH to vaginal secretions, the presence of clue cells microscopically or the character of vaginal secretions.

A [vaginal yeast infection](#) (caused by Candida albicans) is a common cause of vaginitis -- resulting in a white, cheesy discharge; severe [itching](#); [painful intercourse](#); and a [rash](#) or vaginal inflammation.

II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING

- A. Vaginal swab or urethral swab, swabs should be placed in a tube containing 0.5 to 1 mL of saline. Penile discharge and urethral-mucosa scrapings are all acceptable for wet prep examination. Specimens should be collected in a tube containing 0.5 to 1 mL of sterile saline. Slides should be made within 2 hours of blood collection.
- B. Specimens are considered STAT for processing. It is very important that specimens to be examined for Trichomonas vaginalis be delivered to the laboratory as soon as possible and examined within 1 hour after collection. After 1 hour, organisms will lose their motility, particularly when they begin to dry out.
 1. **NOTE:** For specimens that arrive after an hour and are delayed in observation, examine the specimen looking for residual cells that are motile or those that are not. If no organisms are observed in this delayed review, report the comment "Specimen delayed beyond 1 hour in transit. No Trichomonas seen microscopically. These results may or may not be accurate because of the delay. Please submit another specimen for confirmation"
- C. Unacceptable specimens: Any dried-out specimen (delayed in transportation)

III. MATERIALS

- A. Cotton tipped swabs (culturette)
- B. Pipette
- C. Prepared saline solution tubes
- D. Glass slides and coverslips

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- E. Microscope
- F. 10% KOH

IV. QUALITY CONTROL

Illustration chart with pictures of Trichomonas, Yeast, and Positive and Negative Clue Cells. This is to be checked and compared with each specimen tested. Document this in LIS. See Appendix A.

V. PROCEDURE

A. Saline prep

1. Immerse the swab into approximate 0.5 to 1 mL of saline.
2. Gently agitate the swab in the saline to release secretions

B. Microscopic Examination

1. Place a drop of the specimen on a glass slide and add a coverslip.
2. Examine the slide under low power (10x) magnification with low light.
3. Look for motile flagellates. Unless motile flagellates are seen, a diagnosis of Trichomonas vaginalis cannot be made and the slide must be reported as Negative.
4. Suspicious objects must be examined with the high power (40X) objective. Trichomonas vaginalis is slightly larger than a PMN, and you should see flagellar movement.
5. Observe for clue cells (squamous epithelial cells covered in bacteria, especially around the periphery) using the (10X) to screen the slide for epithelial cells and the (40X) objective to identify clue cells.
6. Observe for yeast using the (40X) objective, screen for yeast cells, hyphae and pseudohyphae. If specimen is very cloudy and under microscope exam there is a lot of debris a KOH prep may be performed to verify the presence of yeast.
 - a. KOH Prep
 - 1) Add one drop of specimen to a slide
 - 2) Add a few drops of KOH to the slide.
 - 3) Allow to sit for 2-3 minutes to clear.
 - 4) Add a coverslip.
 - 5) Examine under low power for the presence of pseudohyphae and budding yeast. Verification must be done on 40X.

VI. REPORTING RESULTS

A. Reporting Results

1. The presence/absence of Trichomonas, Clue Cells and Yeast must be reported on all wet preps.
2. Quantitate any Trichomonas, Yeast, or Clue Cells seen on the smear as "Rare, Few, Moderate, or Many"
 - a. Rare: <1-5 organisms or cells per 40X
 - b. Few: 5-10 organisms or cells per 40X
 - c. Moderate: 10-20 organisms or cells per 40X
 - d. Many >20 organisms or cells per 40X
3. If motile organisms are seen, report as "(Quantity) Trichomonas seen on smear".
4. If Yeast are seen on smear, report as "(Quantity) Yeast seen on smear"
5. If Clue Cells are seen on smear, report as "(Quantity) Clue Cells seen on smear".
6. If smear is negative for the organism or cell report as "Absent on smear".

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VII. EXPECTED RESULTS

- A. Trichomonas: Absent on smear
- B. Clue Cells: Absent on smear
- C. Yeast: Absent on smear

VIII. LIMITATIONS

- A. Only wet preparations may be examined. If the specimen has dried on the swab, the organism is no longer viable, and diagnosis cannot be made.
- B. If the specimen is left at room temperature or held at refrigerator temperature for a prolonged period (usually >1 hour), the organisms will lose their motility and die.
- C. Wet mounts have been reported to detect *Trichomonas vaginalis* in 75 to 85% of infected patients. Alternative diagnostic methods may include culture, monoclonal antigen detection kits, permanent stained slides, and collection of a second sample for examination.
- D. If the patient has a *Trichomonas hominis* intestinal infection and the urogenital specimen becomes contaminated with fecal material, a false-positive *T. vaginalis* result may be reported because *T. hominis* and *T. Vaginalis* are similar in shape.

IX. CRITICAL DETERMINANTS

- A. Saline should be checked before addition of the specimen to make sure it is clear of any visible contamination. If saline is not clear and free of visible contamination, discard and use a different tube

X. REFERENCES

- A. Henry, John, 1996. Clinical Diagnosis and Management by Laboratory Methods, 20th ed., pp. 1264-1265. W. B. Saunders Co., Philadelphia
- B. Bailey and Scott, 1998. Diagnostic Microbiology, 10th ed., pp. 372-373,666,829. The C.V. Mosby Co., St. Louis
- C. Henry D. Isenberg, Clinical Microbiology Procedures Handbook, second edition 2004, volume 2, Urogenital Specimens Direct Saline Mount, 9.6.6.1.

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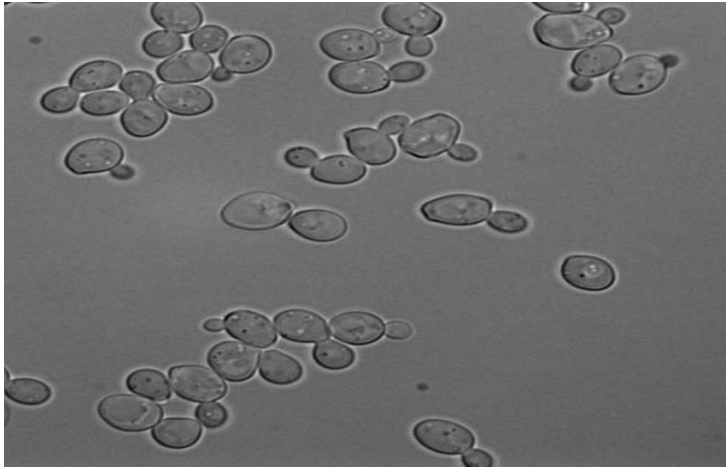
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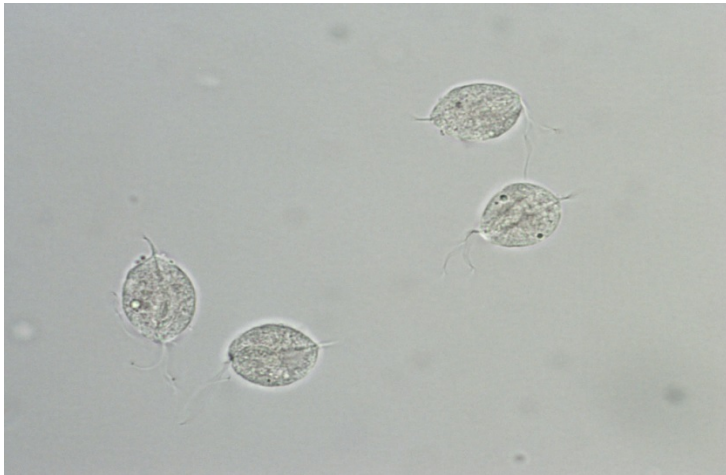
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Appendix A



Yeast



Trichomonas-flagella, motile

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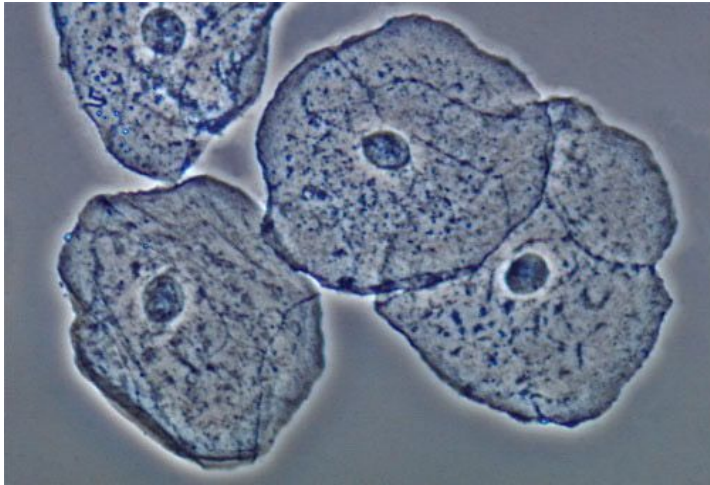
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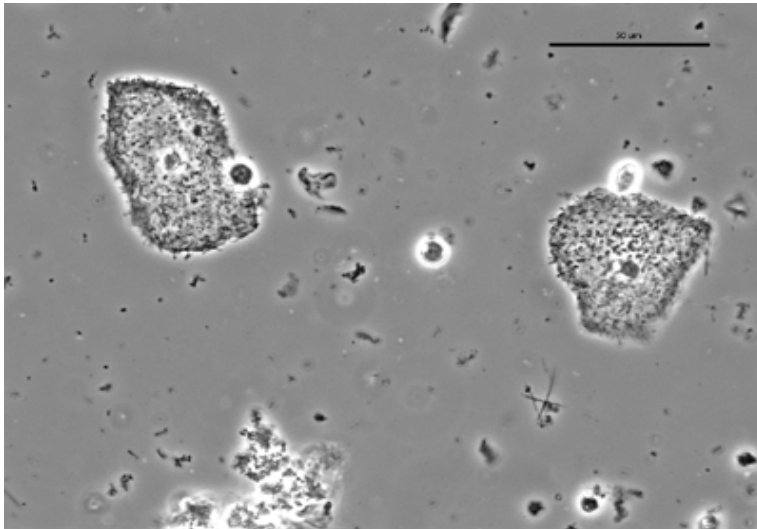
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Epithelial Cells-Normal



Clue Cells-stippled fuzzy edge

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