

## EDUCATIONAL COMMENTARY – BASIC VAGINAL WET PREP

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### Learning Outcomes

On completion of this exercise, the participant should be able to

- describe characteristics used to identify *Trichomonas vaginalis* in a vaginal wet prep;
- describe characteristics used to determine whether an epithelial cell is a clue cell;
- describe how to differentiate small yeast cells from red blood cells; and
- list the advantages and disadvantages of the vaginal wet prep.

### Basic Vaginal Wet Prep

In an age of increasingly complex laboratory testing, certain “low-tech” laboratory tests remain valuable to the clinician. The basic vaginal wet preparation or vaginal “wet prep” used to check for the presence of *Trichomonas vaginalis*, yeasts, and clue cells is one such test.

In the 17th and 18th centuries when Antonie van Leeuwenhoek and Robert Hooke began to explore the microscopic world with the first microscopes, they already had the tools to perform vaginal wet preps. The equipment required consists of a light microscope, glass slides, coverslips, and saline. This test can be done in the physician office laboratory as well as in the most modern hospitals. It can be performed in a timely manner and with minimal equipment.

Although the basic vaginal wet prep can be performed quickly with readily available materials, it requires personnel trained in microscopy. The vaginal wet prep also has the disadvantage of lacking sensitivity and specificity. It is not useful in diagnosing vaginitis caused by some pathogens such as herpes simplex virus. Other tests that are valuable when done in conjunction with the vaginal wet prep include the pH of the discharge and the “whiff test.” The appearance of the discharge is also helpful in determining the diagnosis.

Vaginitis or vulvovaginitis may be caused by a variety of microbial organisms. The most common sources of vaginitis include yeast (predominantly *Candida albicans*), *Gardnerella vaginalis*, and the parasite *Trichomonas vaginalis*, all of which can be observed using basic light microscopy. Bacterial vaginosis

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caused by *G vaginalis* is diagnosed by the presence of “clue cells,” vaginal candidiasis by identifying budding cells or long pseudohyphae, and trichomoniasis by observing motile *T vaginalis* trophozoites.

### Vaginal Wet Prep Procedure

Materials required include cotton or synthetic-tipped swabs, 0.5 to 1 mL of 0.85% saline, microscope slides with coverslips, and a light microscope with 10X and 40X objectives.

Vaginal discharge is collected on a swab and placed into a test tube containing between 0.5 and 1 mL of 0.85% saline. The sample is not stained or mixed with any reagent. The sample should be examined within 1 hour of collection. One drop is placed onto a glass microscope slide and covered with a coverslip. The slide is examined on low (10X) power under low light. The 40X objective may be used to confirm the presence or absence of *T vaginalis*, *G vaginalis*, and yeasts. An alternative technique is to add a drop of saline to a slide and add a small amount of discharge. The slide is covered with a coverslip and examined using the 10X and 40X objectives. It may be helpful to gently warm the sample to increase the motility of *T vaginalis*.

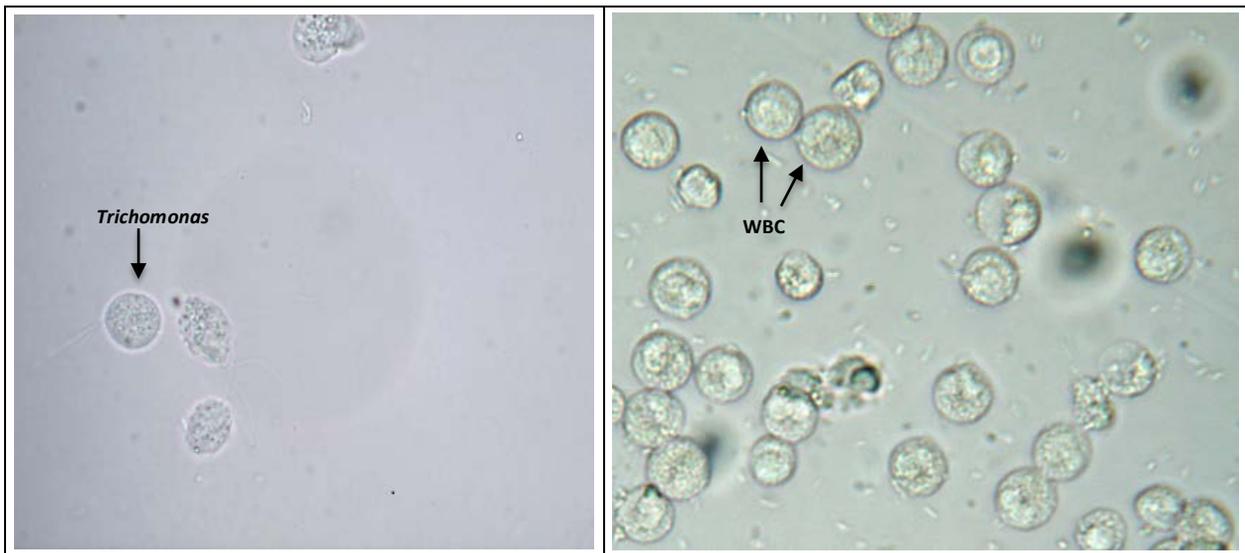
### *Trichomonas vaginalis*

*Trichomonas vaginalis* is a single-cell, flagellated parasite that replicates by binary fusion. It has no known cyst stage. It resides in the female lower genital tract and in males in the urethra and prostate, and is spread by sexual intercourse. Its only known host is humans. *T vaginalis* is identified by detecting motile flagellates. *T vaginalis* is slightly larger than a white blood cell and should have flagellar (axostyle and undulating membrane) motility. If motility is present, the sample may be reported as positive for the presence of *T vaginalis*. It is important to examine the sample as soon after collection as possible, as the organism will lose its motility with delay. It has been determined that motility is present 100% of the time at 30 minutes, 99% at 1 hour, and decreases 3% to 15% for each hour thereafter. The sensitivity of the test also depends on the amount of organisms in the sample. When the organism is no longer motile, it “rounds up,” internalizing the flagella and becoming indistinguishable from a white blood cell (**Figure 1**). This organism is highly susceptible to drying, so a sample received on a slide without saline should be rejected. Because the trophozoite of *Trichomonas hominis* resembles that of *T vaginalis*, any sample contaminated with stool should also be rejected.

*Trichomonas vaginalis* causes a vaginitis that is characterized by a purulent vaginal discharge in women. It is often asymptomatic in men. The sequelae of *T vaginalis* infection may include complications in pregnancy, cervical cancer, and a predisposition to infection with human immunodeficiency virus (HIV). Treatment often consists of metronidazole or tinidazole.

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Other diagnostic testing for *T vaginalis* includes culture, molecular methods, direct fluorescent antigen (DFA) tests, and antigen-detection tests. Culture methods are highly sensitive, but it can take up to a week for results to be available. Molecular tests are also highly sensitive and specific but are usually expensive and require specialized equipment. Direct fluorescent antigen testing also requires specialized equipment and specially trained personnel. Along with the vaginal wet prep, antigen-detection tests are better suited for physician office laboratories. For the detection of *T vaginalis*, the vaginal wet prep has a sensitivity of approximately 60% and specificity of 100%.



**Figure 1. *Trichomonas vaginalis*.** The characteristic twisting, rotating appearance is highly specific for *T vaginalis*. It is important to keep the time from collection to testing as short as possible: ideally, within 1 hour of collection. As the sample ages, the motility of the organism decreases. Once the *Trichomonas* organism is no longer motile, it cannot be distinguished from a white blood cell.

### ***Gardnerella vaginalis***

*Gardnerella vaginalis* is an anaerobic, gram-positive coccobacillus. Because of its thin cell walls, *G vaginalis* often stains as gram-variable. It may be present at low levels in normal vaginal flora. When the normal aerobic vaginal flora, such as *Lactobacillus*, are disrupted, the anaerobic bacteria may take over leading to bacterial vaginosis. Bacterial vaginosis is characterized by thin, gray, homogeneous discharge. The presence of *Gardnerella vaginalis* can be determined by the presence of clue cells in the vaginal wet prep. Clue cells are large, squamous epithelial cells covered with small bacilli. The affected cells will have a fuzzy, grainy, or peppery appearance, and the border of the cell should be obscured by the attached bacteria. Count the total number of distinguishable epithelial cells to determine if a wet prep is to be considered positive for clue cells. To be counted as an epithelial cell the entire cell must be seen and a nucleus must be present. The number of clue cells present should also be counted and that number divided by the total number of epithelial cells; the result should be multiplied by 100 to determine

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the percentage of clue cells. According to Amsel's criteria for the diagnosis of bacterial vaginosis, one criteria is that at least 20% of the cells in the microscopic field must be clue cells. The other three criteria include: Homogeneous appearance of discharge, pH greater than 4.5, and fishy amine odor when mixed with potassium hydroxide (whiff test).



**Figure 2. *Gardnerella vaginalis*.** Bacterial vaginosis may be diagnosed by the presence of clue cells. A clue cell is an epithelial cell in which the edges are obscured by the overlying bacteria. A wet prep is considered positive when more than 20% of the epithelial cells present in the microscopic field are clue cells. It may be tempting for inexperienced practitioners to call any epithelial cell with adherent bacteria a clue cell. The criterion of not being able to see a well-delineated edge on the epithelial cell should be stressed.

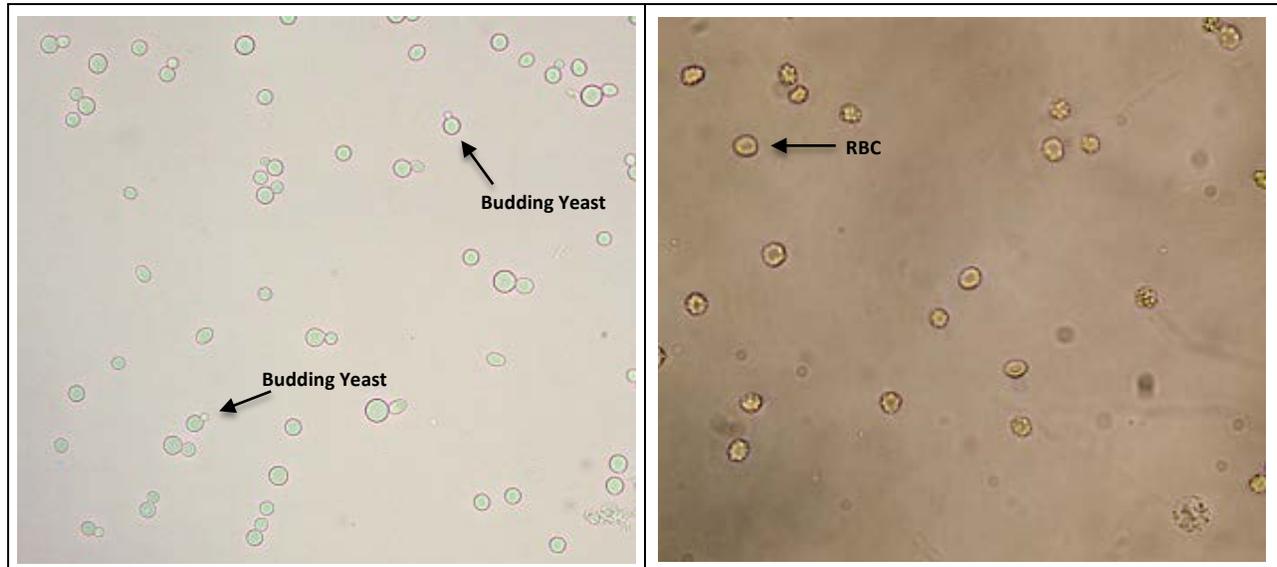
Another laboratory test available to aid in the diagnosis of bacterial vaginosis is culture. Culture is not optimal as it may delay in treatment. Bacterial vaginosis may lead to complications such as premature delivery, sexually transmitted infections such as HIV, and pelvic inflammatory disease. Treatment options include metronidazole. Bacterial vaginosis itself is not considered a sexually transmitted disease.

### Candida/Yeast

The presence of yeast in predominant amounts within the vaginal wet prep is considered positive for candidiasis. The yeast may appear as oval budding yeast or yeast showing a chain of cells known as *pseudohyphae*. It may be difficult to differentiate some yeast cells from red blood cells. To differentiate red blood cells from yeasts, the examiner should focus up and down using the 40X objective to observe the cell in question in different dimensions. Red blood cells have a biconcave appearance, which yeast

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cells do not. Red blood cells also have a consistent size of 6 to 8  $\mu\text{m}$ , whereas yeast cells can vary greatly in size. Usually, single yeast cells range 3 to 7  $\mu\text{m}$ .



**Figure 3. Candidiasis.** It is estimated that 75% of women will have at least one yeast infection during their lifetime. Candidiasis is caused by an overgrowth of yeast such as *C albicans*. It is visualized on the vaginal wet prep as small oval budding yeast or long chains of pseudohyphae. Red blood cells may be differentiated from yeast cells by focusing up and down through several planes. Red blood cells appear biconcave; yeast cells have a uniform appearance.

Vaginal candidiasis is most often caused by the yeast *Candida albicans*, though other species of yeast have been indicated as a cause. A small amount of yeast may be present as part of the microflora of the vagina. Candidiasis can be caused by changes in the normal vaginal flora due to antibiotic use; uncontrolled diabetes; pregnancy; an impaired immune system; or hormonal changes from birth-control use, menopause, or pregnancy. Discharge is often described as cottage cheese-like. As with bacterial vaginosis, candidiasis not considered a sexually transmitted disease. Complications from candidiasis are rare.

### White Blood Cells

The presence of an elevated number of white blood cells is considered to be a sign of inflammation. Moderate inflammation is indicated by a ratio of five WBCs for every one epithelial cell. Moderate to severe inflammation is indicated by a ratio of >10 WBCs for every epithelial cell. An increased number of WBCs is common with *Trichomonas vaginalis* but not in bacterial vaginosis.

### Conclusion

In conclusion, some of the challenges in performing the vaginal wet prep include technical expertise, collection technique, time from collection to testing, and sensitivity and specificity. Advantages of the

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basic vaginal wet prep include low cost, timeliness, and no need for specialized equipment. Alternative testing modalities, such as molecular methods, special stains, or cultures, can be time-consuming as well as expensive and may not be available to physician office laboratories or small hospital laboratories. The vaginal wet prep is sure to remain a useful diagnostic tool.

### References

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