

**Wet Mount Vaginal Preparation Procedure**  
**Department of Microbiology**

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## 1.0 Clinical Significance

Bacterial vaginosis (BV) is a clinical syndrome characterized by an abnormal vaginal discharge in women in childbearing years accompanied by a rise in pH from 4.5 and an amine (fishy) smell. The microbiota of the vagina shifts from predominantly lactobacilli to a mixture of *Gardnerella vaginalis*, *Prevotella* spp., *Mobiluncus* spp., and often other anaerobes and *Mycoplasma hominis*. Bacterial vaginosis has been associated with preterm birth, miscarriage, amniotic infections, and postpartum endometritis.

Vaginitis is an inflammation of the vagina that can result in discharge, itching and pain. Vaginitis is frequently caused by a change in the normal balance of vaginal microbiota. Yeast infection occurs when there is an overgrowth of yeast, usually *C. albicans*. Candidiasis may be associated with predisposing factors such as, pregnancy, birth control, diabetes, broad-spectrum antimicrobial therapy, steroid therapy and immunosuppression. Vaginitis may also occur as the result of a sexually transmitted infection, most commonly *Trichomonas vaginalis*. Women with trichomoniasis are at a greater risk of acquiring HIV and other sexually transmitted infections.

Pinworm infection is the most common type of intestinal worm infection in the United States. Infection occurs most often in school age children and the microscopic eggs are easily spread from child to child or to other family members. Occasionally, the parasite will invade the female genital tract and cause vulvovaginitis.

## 2.0 Principle

Evidence of BV and vaginitis may be seen microscopically. Yeast may appear as either budding cells or pseudohyphae. *Trichomonas vaginalis* are protozoa that are recognized by their characteristic jerky motility. While clue cells are frequently evident in wet mount preparation of vaginal samples from patients with BV, a Gram stain is a more sensitive and specific technique that permits scoring of the relative amounts of microbial morphotypes. Pinworm eggs can also be found in vaginal smears, and adult worms may be seen during vaginal examinations.

## 3.0 Scope

This procedure is classified under CLIA as highly complex. It should be carried out by technical personnel familiarized and trained to perform microscopic examination of clinical specimens.

## 4.0 Safety - Personal Protective Equipment

Performance of this procedure may expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

**This procedure may expose you to:**

- Bloodborne pathogens

**To perform this procedure, you must use:**

- Gloves must be worn when handling specimens.
- A laboratory coat must be worn when handling specimens.

**Disinfectant following procedure:**

- Bleach dilution sprayers can be used for on demand disinfectant.

## 5.0 Specimen Requirements

### 5.1 Specimen Collection, Handling and Storage

1. Collect vaginal fluid/discharge from the posterior fornix using a sterile swab.
2. Place the swab in transport medium (e.g., Amies gel, liquid Stuart's or eSwab), and transport at room temperature.
3. Specimens must be examined within 1 h for trichomonads.

## 5.2 Specimen Rejection Criteria

Specimens should be rejected: if swabs are submitted without transport medium, if they are received > 2 h after collection, or if the specimen was refrigerated or frozen.

## 6.0 Materials

### 6.1 Consumables

- Transfer pipettes
- Glass test tubes
- Glass microscope slides
- Coverslips

### 6.2 Reagents

- Sterile saline

## 7.0 Procedure

### 7.1 Specimen Preparation

#### Spun-fiber Swabs

1. Place a small accession label on the frosted end of a glass microscope slide. Prepare a smear for Gram stain by pressing and rolling the swab against the center of the slide. Refer to the Gram Stain Procedure for staining instructions.
2. For the wet mount, suspend the specimen in 3-4 drops of sterile saline in a sterile glass tube. Place 1 or 2 drops of specimen suspension onto the slide and add coverslip

#### Flocked Swabs in Liquid Amies (eSwab)

1. Vigorously shake the ESwab tube containing the swab sample between the thumb and forefinger for 5 s or mix the tube using a vortex for 5 s to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the liquid transport medium.
2. Place a small accession label on the frosted end of a glass microscope slide. Prepare a smear for Gram stain by transferring 1 or 2 drops of the suspension onto the center of a glass slide. In case of bloody or thicker specimens, care should be taken to thinly spread the sample on the slide. Refer to the Gram Stain Procedure for staining instructions.
3. For the wet mount, place 1 or 2 drops of specimen onto the slide, and add coverslip.

### 7.2 Wet Mount & Gram Stain Examination

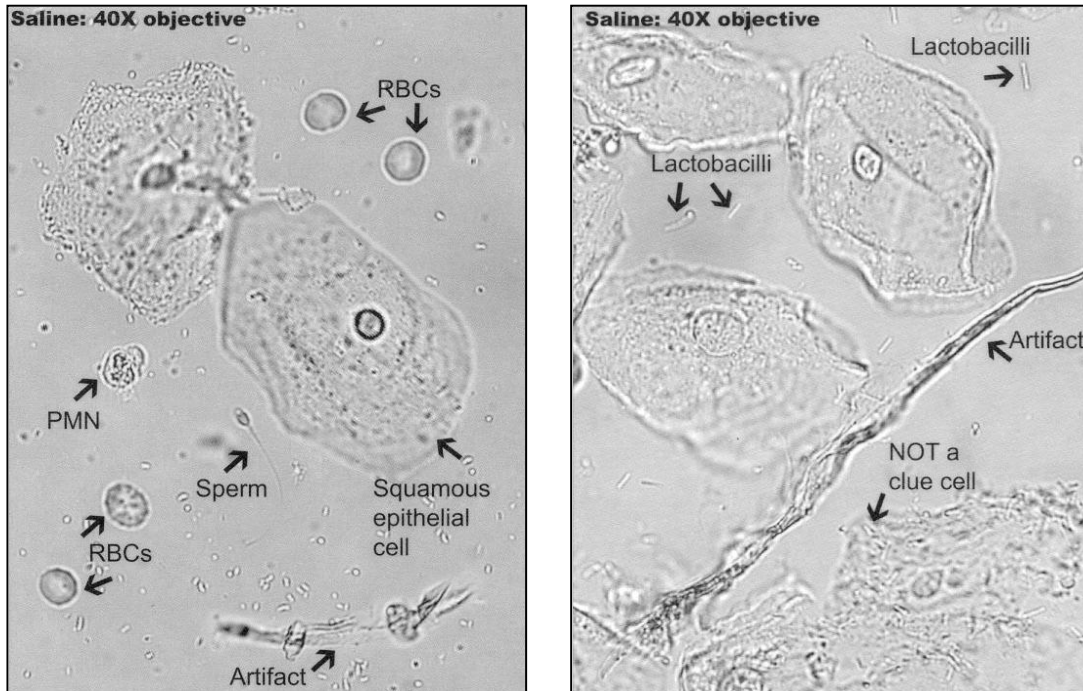
1. Adjust the microscope lighting to better visualize unstained cellular elements.
2. Examine the wet mount preparation using the low (10X) objective. Scan the prep for signs of motile trichomonads or pseudohyphae. Examine suspicious items on high dry (40X) for confirmation.
3. Examine the prep on high dry for trichomonads and budding yeast. Weakly motile *Trichomonas* may only be evident on high power.
4. After examining the wet prep, examine the Gram stain for evidence of BV.

## 8.0 Interpretation & Reporting of Results

### 8.1 Cellular Elements Seen in Vaginal Secretions

Cellular elements that are typically seen in vaginal wet mount preparations include squamous epithelial cells, neutrophils (PMNs), red blood cells (RBCs), and occasionally sperm cells. RBCs are small, concave discs about 7 µm in diameter and have no interior detail. Neutrophils are small and round with a multi-lobed nucleus in the cytoplasm. Neutrophils are 15 µm in diameter. Squamous epithelial cells are large, flat cells (30 to 50 µm) with a small, single nucleus and a large area of cytoplasm. They may appear as single cells or grouped in clusters or sheets. Some cells are flat with well-defined edges, or they may be folded, or rolled. The nucleus is about the size of a small neutrophil. There may be some granularity in the cytoplasm. Bacteria

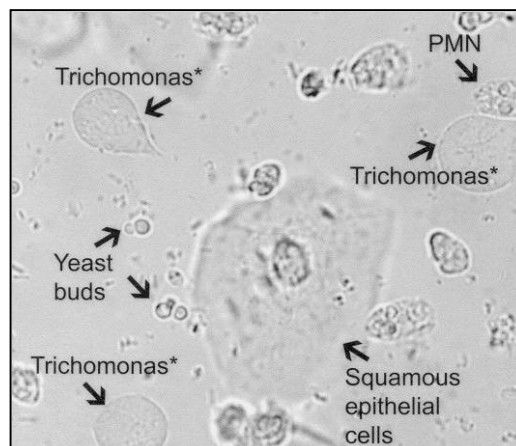
are usually seen. Lactobacilli typically appear as long slender rods. A variety of artifacts may be encountered in vaginal wet preparations, including fibers, pollen grains, spermatozoa, starch granules, mucous strands, and other artifacts. Examples of cellular elements and artifacts are shown below.



## 8.2 *Trichomonas vaginalis*

*Trichomonas vaginalis* cells have 3 to 5 anterior and 1 posterior flagella. They are about 20 µm in diameter. They have rapid, jerky motility that can be seen under low power microscopy, although weak motility may only be evident on high power. Their characteristic motion causes cells around them to move, as well. Organisms must be motile in order to be reported. Because the numbers of trichomonads vary in clinical specimens, it is important to scan the entire slide. A second technologist must verify all positive results and documentation of the verification must be entered into the workup.

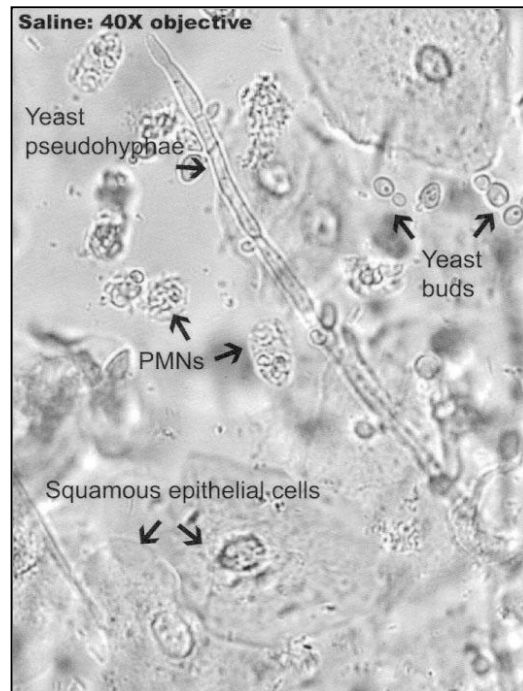
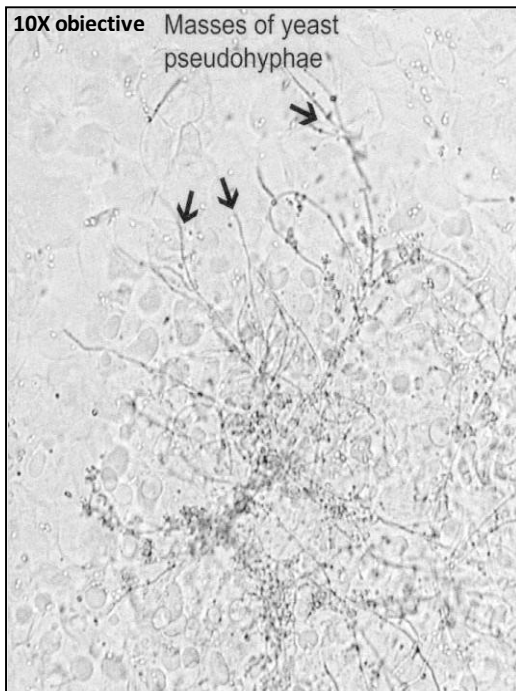
Report either, ***Trichomonas* seen [TRCH]** or **No *Trichomonas* seen [NTRCH]**. If the specimen was collected > 1 h prior to examination and no trichomonads are seen, add the following comment: **Delay in transit decreases the ability to detect motile *Trichomonas*. If testing for *Trichomonas* is indicated please order Vaginal Pathogens by DNA Probe (VPDNAP) or *Trichomonas* by amplification assay (APTTV). [DETR]**



### 8.3 Yeast

There are two morphologic forms that are seen in clinical specimens, the yeast phase (blastoconidia) and pseudohyphae. A single yeast cell is 5-7  $\mu\text{m}$  in diameter and can be seen with or without a bud. Single yeast cells or yeast buds are difficult to visualize using a 10X objective but can be easily confirmed using a 40X objective. *Candida* spp. can form elongated cells called pseudohyphae up to 50  $\mu\text{m}$  long. Pseudohyphae appear as thick-walled tube-like structures, appear branched, and may have terminal buds. Pseudohyphae can be visualized using a 10x objective but should be confirmed using 40X objective.

Report either, **Yeast seen [YSTS]** or **No yeast seen [NYS]**.



### 8.4 *Enterobius vermicularis* (Pinworm)

Pinworm eggs are elongated, measuring 50-60  $\mu\text{m}$  by 20-30  $\mu\text{m}$ . They are smooth and flattened on one side. The eggshell is colorless and a folded embryo is visible inside.

The adult female worms are 8-13 mm long by 0.3-0.5 mm wide and have a long pointed tail. The adult male worms are 2.5 mm long by 0.1-0.2 mm wide and have a blunt posterior end.

Report as, ***Enterobius vermicularis* (pinworm) [ENVL]**. Do not report the absence of pinworm for routine vaginal preparations since it is an incidental finding.



### 8.5 Gram Stain for Bacterial Vaginosis

Evidence of BV should be determined by the scored Gram stain rather than reporting the presence or absence of clue cells in the wet mount preparation. Using the score chart for bacterial vaginosis, determine a value of 0 to 4+ for each of the 3 organism morphologies. Add the 3 values to obtain the total score. Photomicrograph examples of the various organism morphologies can be found in the Gram Stain Procedure.

### Bacterial Vaginosis Score Chart

+		+		+	
Lacto	Score	<i>G. vag/ Bacteroides</i>	Score	Curved GNR	Score
4+	0	4+	4	3 to 4+	2
3+	1	3+	3	1 to 2+	1
2+	2	2+	2	0	0
1+	3	1+	1		
0	4	0	0		
<u>Quantitation</u>		<u>Total Score</u>	<u>Interpretation</u>		
4+ = >30 orgs/OIF		0 to 3	Smear is not suggestive of bacterial vaginosis.		
3+ = 6 to 30 orgs/OIF		4 to 6	Altered vaginal flora, hold smear for review.		
2+ = 1 to 5 orgs/OIF					
1+ = < 1 orgs/OIF					
0 = no orgs/OIF		7 to 10	Smear is suggestive of bacterial vaginosis.		

If the score is 0 to 3, report: Smear not suggestive of bacterial vaginosis. [BVNEG]

If the score is 4 to 6, report: Altered vaginal flora. [BVINT].

If the score is 7 to 10, report: Smear suggestive of bacterial vaginosis. [BVPOS]

## 9.0 Quality Control & Quality Assurance

Positive controls are not necessary for vaginal wet mounts. However, competency is assessed annually using the Vaginal Wet Prep competency assessment tests on [www.medtraining.org](http://www.medtraining.org). For *Trichomonas*, a second technologist must verify all positive results and documentation of the verification must be entered into the workup.

## 10.0 Limitations

1. Failure to vigorously swirl spun-fiber swabs in the saline to dislodge the specimen may lead to false-negative results.
2. Non-motile *Trichomonas* could be mistaken for white blood cells.
3. Oil droplets from intravaginal medications may be mistaken for yeast. However, oil droplets vary greatly in size and are highly refractile.
4. Cotton fibers from the swab may resemble fungi.
5. Failure to examine the entire cover slip area may result in false negatives.
6. There are noninfectious causes of vaginitis. Vaginal sprays, douches, perfumed soaps, scented detergents, and spermicidal products may cause an allergic reaction or irritate vulvar or vaginal tissues. Thinning of the vaginal lining, related to decreased hormone levels following menopause or surgical removal of ovaries, can also cause vaginal itching and burning.

## 11.0 References

1. Garcia, L.S. 2007. *Clinical Microbiology Procedures Handbook*, 3<sup>rd</sup> ed., Vol. 2. ASM Press, Washington, D.C.
2. Versalovic, J, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock. 2011. *Manual of Clinical Microbiology*, 10th ed., Vol. 1, ASM Press, Washington, D.C.
3. Seattle STD/HIV Training Center. Vaginal Wet Preps training reference.

4. [www.medtraining.org](http://www.medtraining.org) Vaginal Wet Prep training module.
5. [www.mayoclinic.org](http://www.mayoclinic.org) Diseases and Conditions – Vaginitis.

## 12.0 Document Control History

Medical Director Approval: Dr. Joseph Schappert 03/10/2010

Microbiology Director Approval: Dr. Ann Robinson 02/02/2006, reviewed 01/27/2015

Microbiology Supervisor Reviews: Jerry Claridge 02/01/2006, 01/2007, 09/2007, 09/2008, 09/2009, 03/2011, 03/2013, 01/27/2015

Revisions & Updates: 01/27/2015 Updated for eSwabs, added sections for clinical significance, safety, QA, and limitations. Added images. Added GS confirmation for preps without clue cells. Added information for incidental pinworm findings. 02/12/2015 Removed protocol for calling clue cells from the wet mount and added instructions for performing scored Gram stain. Due to the lack of specificity associated with the wet mount, evidence of BV should be determined from a scored Gram stain.