



## County Health Department Laboratory Procedures

Title: Vaginal Wet Prep Procedure

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# VAGINAL WET MOUNT

## INTRODUCTION

The diagnosis of initial vulvovaginal discharge complaints is usually straightforward, and in a majority of cases the diagnosis will be bacterial vaginosis (BV; 70% of infectious cases), vulvovaginal candidiasis, or trichomoniasis. Specific infections are frequently associated with distinctive vaginal discharge:

- A vaginal yeast infection often causes a white, lumpy discharge that looks like cottage cheese.
- Trichomoniasis causes a vaginal discharge that is yellow-green, foamy, and bad-smelling.
- BV generally produces a vaginal discharge that is thin and milky with a strong fishy odor.

Although the most common forms of vaginitis and vulvitis are BV, trichomoniasis and candidiasis, more causes exist, many of them noninfectious. Patients with recurrent and resistant infections pose a greater diagnostic challenge. Most challenging are patients with a symptomatic problem without evidence of any of the common causes.

While it is often possible to define the cause of a vaginal discharge based on the patient's history and/or physical exam, an algorithmic approach, incorporating symptoms, signs, vaginal pH, and microscopy, can lead to a more accurate diagnosis.

## PRINCIPLE

A vaginal wet mount preparation (or vaginal smear or wet prep) is a gynecologic test wherein a sample of vaginal discharge is observed by wet mount microscopy by placing the specimen on a glass slide and mixing it with 0.9% normal saline or 10% potassium hydroxide (KOH). Normal saline is a physiologic solution, so cell membranes are preserved and vital activities (movement of protozoa, sperm, etc.) are undisturbed. Saline is the best solution for visualizing motile trichomonads in cases of trichomoniasis, and the bacterial studding of vaginal epithelial cells, known as "clue cells", that are characteristic in cases of BV. When a yeast infection (*Candida*, *Monilia*) is suspected, the addition of 10% KOH to the vaginal sample can provide a clearer view of yeast buds and pseudohyphae. KOH will hydrolyze bacterial cells, trichomonads, red and white cells and, to a certain extent, epithelial cells but will leave yeast intact, making it particularly useful in identifying yeast in vaginal discharge. In many cases of BV or trichomoniasis, addition of KOH to the vaginal discharge will produce a strong amine or fishy smell. The latter procedure is called a "Whiff" test.

Vaginal wet preparation testing demands a certain level of technical skill and training to be performed properly. According to the Clinical Laboratory Improvement Act (CLIA), it is considered as a subcategory of the "moderate complexity" level. Vaginal wet prep and a limited set of other microscopic evaluations on labile specimens (e.g., urine, skin scrapings and excretions) performed by healthcare providers during patient office visits are listed in the CLIA regulations. These examinations were first described as "physician performed microscopy" but are now called "provider performed microscopy" (PPM). PPM may only be

performed by a suitably credentialed physician, dentist or a midlevel practitioner (e.g., physician's assistant, nurse practitioner).

## **SPECIMEN COLLECTION**

### **Indications**

A vaginal wet mount may be considered in cases of vaginitis symptoms, such as vaginal itching, burning, rash, odor, or discharge. It may assist in suspicion of vaginal yeast infection, trichomoniasis and BV.

### **Counter Indications**

A vaginal wet mount is not done during the menstrual period, because menstrual blood on the slide can confound the results. It is preferred that the patient not douche within 3 days prior to sample collection. Also, tampon use or having sex can potentially disrupt the normal vaginal pH and should be avoided for 24 hours before the test. Vaginal medicines (such as a nonprescription vaginal yeast medicine) should not be used 2 to 3 days before the test.

### **Collection Procedure**

The sampling procedure may cause some discomfort and minor bleeding, but otherwise there are no associated risks.

1. The patient is positioned in lithotomy position on the exam table (as for a pelvic examination).
2. Use a spatula or cotton- or synthetic-tipped swab to obtain a sample of vaginal discharge. The fluid specimen can be obtained directly from the mucosa of the posterior vagina or the vaginal secretions. Also, usually there is abundant discharge on the inside curve of the speculum after it is removed.

Note: Use of lubricants should be avoided since they may be toxic to trichomonads and/or cause interference with the interpretation of the preparation. Collection of an inadequate volume of vaginal discharge may result in false negative findings.

3. Do not allow the specimen to dry on the swab (or slide).
4. Make a slide preparation of the specimen (see procedure below).

## **MATERIALS AND EQUIPMENT**

- Speculum
- Spatula
- Sterile, cotton- or synthetic-tipped swabs
- 0.85-0.9% sterile saline
- 10% KOH
- Glass or plastic test tube
- Clean glass microscope slides
- 22mm x 22mm glass coverslips
- Compound microscope

- Lens cleaning solution and lens paper

Normal saline (0.9%) and KOH (10%) solutions are obtained from the OSDH Pharmacy. All reagents are labeled by the manufacturer and require no further preparation. Store saline and KOH solutions at room temperature. All laboratory reagents must be labeled with the date of receipt, date opened, and date of expiration. Do not use passed expiration date. Do not use solutions if they appear cloudy or contaminated or have precipitates.

### SAFETY PRECAUTIONS

Universal Safety Precautions are required for all laboratory testing. Refer to the *Oklahoma State Department of Health Infection Prevention Control Manual: Section 3: Infection Prevention Practices; Section 4: Guidelines for Cleaning, Disinfecting and Sterilizing; and Section 5: Management of Regulated Medical Waste.*

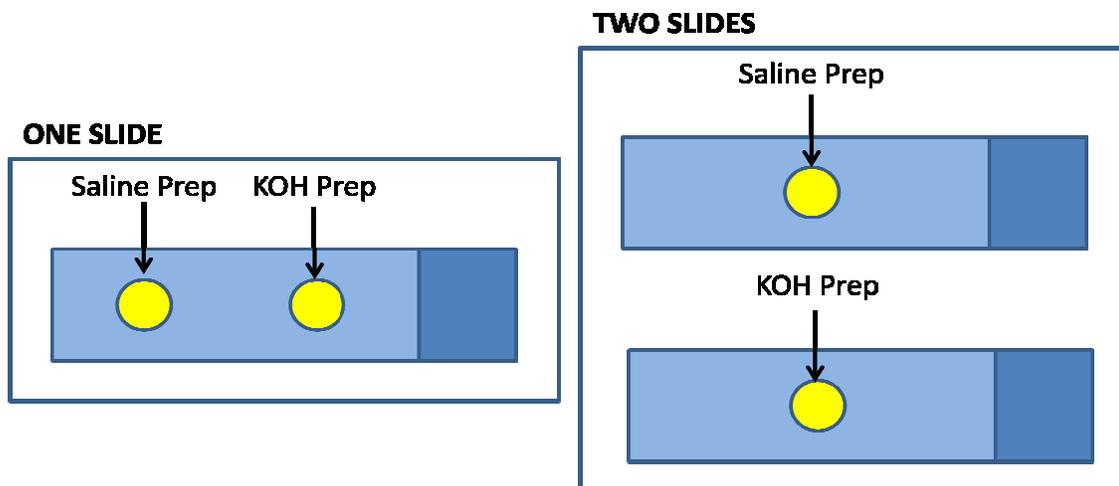
### WET MOUNT PROCEDURE

#### Preparing a Wet Mount Slide of Vaginal Discharge

Wet mount slide preparations of vaginal discharge are prepared ***one patient at a time and examined microscopically immediately following specimen collection.*** Specimens must ***not*** be stored and/or batched with other patient specimens prior to examination.

It is important to work quickly and not to allow the preparations to dry on the slide. Also, in order to see movement of trichomonads, the preparations must be as fresh as possible; examine slides immediately following preparation, otherwise collect a fresh specimen.

A saline wet preparation is prepared on each patient. A KOH wet preparation may be prepared as clinically indicated in conjunction with the saline wet preparation. The saline and KOH wet preparations may be prepared on a single microscopic slide or on two separate slides, at the discretion of the practitioner (see figure below). When using one slide for both specimens, it is important to keep the specimens far enough apart that there will be no mixing of the NaCl and KOH.



Labeling of the slide(s) is not necessary since the slide(s) will be read immediately following preparation.

Wet mounts of vaginal discharge may be prepared using either the *direct* (saline or KOH added directly to discharge on slide) or *indirect* (discharge added to saline in tube then mixture added to slide) methods:

#### **Direct Method**

1. Using a sterile, cotton- or synthetic-tipped swab, directly apply a very small portion of the freshly collected vaginal discharge to each end of a glass microscope slide (or to separate glass slides). One portion of the specimen will be used for the saline preparation and the other portion will be used for the KOH preparation. Do not use too much discharge, since it will make it harder to evaluate the slide.
2. Add one drop of saline to one of the specimens on the slide, then mix, coverslip and examine microscopically (see **Saline Wet Prep** below).
3. One drop of KOH will be added to the other specimen on the slide, then mixed coverslipped and examined microscopically (see **KOH Prep** below).

#### **Indirect (Saline Tube) Method**

1. Place the sterile, cotton- or synthetic-tipped swab containing the freshly collected vaginal discharge into a tube containing 1.0 mL of saline.
2. Using the same swab used for collection or a fresh swab or dropper, gently mix the sample with the saline, then apply one drop of the specimen from the tube to each end of the glass microscope slide (or to separate slides).
3. One drop of the specimen is then coverslipped and examined microscopically (see **Saline Wet Prep** below).
4. One drop of KOH will be added to the other specimen, then mixed, coverslipped and examined microscopically (see **KOH Prep** below).

#### **Saline Wet Preparation**

1. Once the vaginal discharge sample has been prepared on the slide by the Direct or Indirect Method as described above, place a coverslip over the specimen to be used for saline wet prep. Avoid the formation of excess air bubbles below the coverslip.
2. Immediately, examine the specimen microscopically using both the low (X10) and high (X40) power objective lenses. (See the *Appendix: Components of the Compound Microscope* for terminology used to describe parts of the microscope)
  - a. Place the slide on the microscope stage so that the edge of the coverslip is in the middle, directly over the beam of light exiting the condenser.
  - b. With the 10X objective lens in the viewing position, use the coarse focus control to raise the stage to the highest position.
  - c. While observing through the eyepieces, use the coarse focus control to gradually lower the stage until the edge of the glass coverslip and fluid beneath it comes into focus.
  - d. Using the mechanical stage control knobs, move the slide to begin observing the saline preparation.

- e. Using the fine focus control knob, make fine focus adjustments to bring epithelial cells and other structures on the slide into clear view.
- f. Finally, adjust the light so that structures on the slide can be viewed clearly.
- g. Begin scanning the slide using the X10 objective lens for evidence of trichomonads (look for movement of these organisms), yeast buds, pseudohyphae, clue cells and other structures important for diagnosis (i.e., white blood cells).
- h. It is important to confirm the identity of structures using the 40X objective lens (turn the nose piece of the microscope to engage the 40X objective lens and make adjustments to the focus using the *fine focus control knob only*) since certain natural components of vaginal fluid specimens and artifacts (e.g., hair, fibers, rod bacteria, bubbles) can be misinterpreted.

### KOH Preparation

A KOH preparation should be made ***immediately after reading the saline wet prep***, as clinically indicated. Adding a drop of 10% KOH solution to a vaginal discharge specimen effectively disrupts the cell membranes of bacteria, trichomonads, WBCs, RBCs, and to a lesser extent epithelial cells, such that they are lysed. Yeast, if present, remains intact due to its thick cell wall; this makes the yeast more easily distinguished in the preparation. However, if left for a prolonged period of time, the yeast too will be lysed by the KOH, so it is important to work quickly.

1. Add a drop of 10% KOH to one of the preparations and mix evenly.
2. Smell the vaginal discharge preparation as the KOH is added; a strong amine or fishy smell is often indicative of a BV or trichomonas infections. If BV or trichomonas infection is suspected, a Whiff test may be performed by adding KOH directly to the sample on the slide or into the tube without subsequent microscopic examination. Note that in some women, it is not necessary to mix vaginal discharge with KOH to obtain a positive "Whiff" test. A highly alkaline vaginal pH or recent sexual intercourse may produce the "fishy odor" without special preparation.
3. Place a coverslip over the specimen. Avoid the formation of excess air bubbles below the coverslip.
4. It is important to allow the KOH to react with the sample for 30-60 seconds before making an observation.
  - a. Using the procedure described above in the *Saline Wet Preparation* section find the plane of focus of the specimen on the slide then begin examining the KOH prep using the 10X low power objective lens.
  - b. Scan the slide using the 10X low power objective lens with reduced lighting. Examine numerous fields for the presence of pseudohyphae and/or budding yeast cells.
  - c. It is important to confirm the identity of suspected fungal elements using the 40X objective lens (turn the nose piece of the microscope to engage the 40X objective lens and make adjustments to the focus using the fine focus control

knob only) since certain natural components of vaginal fluid specimens and artifacts (e.g., hair, fibers, rod bacteria, bubbles) can be misinterpreted.

## Microscopic Observations

The following observations may be made:

- Yeast
  - Note that yeast “buds” must be present for the diagnosis of a yeast infection. Be careful in misinterpreting filamentous artifacts, such as fibers or hairs, as pseudohyphae. Not all yeast species produce pseudohyphae. All pseudohyphae must contain buds to be considered yeast pseudohyphae. Yeast, with their thick cell walls, may be more easily identified on KOH preparations, whereby other cellular material will be lysed in KOH-treated specimens. Low numbers of yeast, in the absence of clinical signs and symptoms, are considered part of the normal vaginal flora.
- Trichomonads
  - Note that the characteristic movement of trichomonads is very helpful in making this positive finding; however, motility will slow and eventually cease as the saline wet preparation ages, so it is important to view the slide immediately to detect these organisms. Do not warm the saline slide if it has cooled but rather collect another sample. There may be very few trichomonads present but the presence of one motile trichomonad is sufficient to call the specimen **positive** for *Trichomonas vaginalis*. The saline wet preparation is only 50% sensitive for detection of trichomonas. Cultures may increase the sensitivity to approximately 90%. The absence of the classical green/yellow, frothy discharge does not exclude trichomoniasis. These structures will be lysed in the KOH-treated specimen but trichomonas infections often produce a positive Whiff test.
- Epithelial cells
  - Epithelial cells should be present in every saline wet prep specimen, otherwise, the specimen is considered unsatisfactory. These structures will be lysed in the KOH-treated specimen.
- Clue cells
  - Note that 20% or more of the epithelial cells must be clue cells for the diagnosis of BV. Also, at least 75% of the edges of an epithelial cell should be obscured by bacteria in order to call it a clue cell; the nucleus may or may not be visible. These structures will be lysed in the KOH-treated specimen.
- White blood cells (WBCs)
  - Note that WBCs play a role in fighting infections and can be elevated in numbers (>10/hpf) in some infections, especially in *Chlamydia*, *Trichomonas vaginitis*, herpes, and GC, but generally not in BV. A ratio of 5 WBCs to every 1 epithelial cell (5:1) indicates possible *mild* inflammation while a ratio greater than 10:1 indicates possible *moderate* to *severe* inflammation. These structures will be lysed in the KOH-treated specimen.
- Red blood cells (RBCs)

- RBCs may be apparent to various degrees in wet preps of vaginal discharge. In fresh preparations, they appear highly refractile as compared to the granular appearance of WBCs and are slightly smaller. In older preparations, the cell membrane may appear crenulated. These structures will be lysed in the KOH-treated specimen.

See examples of these structures in the *Appendix: Examples of Microscopic Details on Wet Mounts*.

### **Recording Wet Prep Results**

Wet prep results are entered on **Form ODH-303L** and placed in the patient's chart.

### **Microscopic Observations**

Microscopic observations are recorded for each of the following structures:

- Clue cells
- Yeast
- Trichomonads
- WBCs
- RBCs

These structures should be quantified using the following notations:

- Negative (–; 0; or N)
  - Negative *Saline Wet Preparations* will show no evidence of yeast, trichomoniasis, or clue cells. WBCs should be few in number. Moderate numbers of bacilli may be present. Epithelial cells must be present; otherwise, the specimen is unsatisfactory.
  - Negative *KOH-treated Wet Preparations* will show digested debris of cells and no evidence of yeast or pseudohyphae. Initially, epithelial cells may appear swollen or enlarged and small cocci may be observed.
- Positive
  - 1+ or + = small amount of items observed
  - 2+ or ++ = moderate amount of items observed
  - 3+ or +++ = large amount of items observed

### **Whiff Test Result**

In addition, the result of the Whiff test should be recorded (as positive (+) or negative (-)) for the KOH-treated specimen.

### **Other Information**

Additionally, the following information is documented on the ODH-303L:

- Type of test (saline or KOH)
- Time of specimen collection/receipt (note: the time of specimen collection and receipt will be the same)
- Time test completed
- Initials of person performing the test.

An example of results recorded for a patient on Form 303L is illustrated below:

WET PREP	Saline X KOH
Time Coll/Rec'd	11:20 am
Yeast	+
Trich	-
WBCs	-
Clue cells	-
RBCs	-
Whiff	-
Time Completed	11:23 am
Initials	LB

WET PREP	Saline KOH X
Time Coll/Rec'd	11:20 am
Yeast	+
Trich	-
WBCs	-
Clue cells	-
RBCs	-
Whiff	-
Time Completed	11:28 am
Initials	LB

## DISPOSAL OF SPECIMENS

Swabs and wet mounts are considered biohazardous. For disposal procedures refer to the *Oklahoma State Department of Health Infection Prevention Control Manual: Section 5: Management of Regulated Medical Waste*. Slides must be stored in puncture-proof biohazardous containers prior to disposal.

## TROUBLESHOOTING PROBLEMS WITH MICROSCOPE OPERATION

### Problem

### Course of Action

Unable to focus

- Adjust coarse and fine focusing knobs
- Check lens for dirt and debris (KOH) build-up
- Check coverslip for air bubbles

Unable to distinguish organisms

- Adjust light source for the necessary amount of light required (Low lighting is recommended)

Unable to locate specimen

- Use procedure outlined in *Saline Wet Prep* section of this procedure to determine the plane of focus

Specimen cloudy/debris present

- Inadequate volume of specimen - test another specimen
- KOH precipitate present – use fresh KOH

Microscope inoperable

- Contact MIH/Family Planning at 271-4476 for information concerning repair/replacement.

## MICROSCOPE MAINTENANCE

See *Appendix: Microscope Preventive Maintenance* for instructions on general care and maintenance of the microscope. Scheduled preventive maintenance is performed on microscopes by a contracted vendor. Scheduled preventive maintenance and any unscheduled repairs are documented in the Equipment Maintenance and Repair Log (see *Appendix: Equipment Maintenance and Repair Log*).

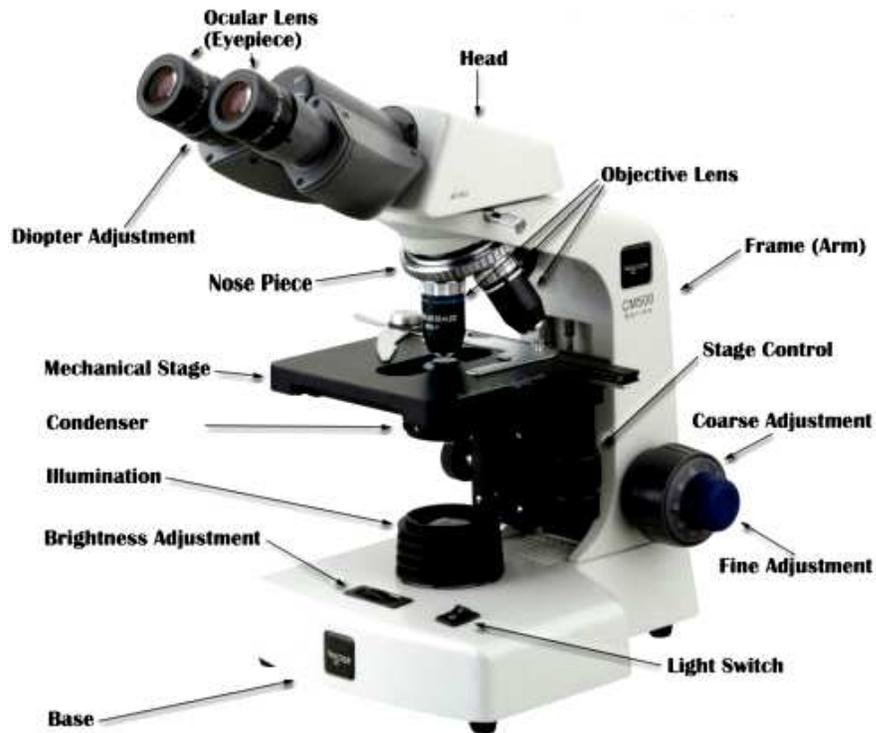
## REFERENCES

1. McGinnis, M.E., Laboratory Handbook of Medical Mycology, Academic Press, Inc., New York, 1980 Ed.
2. Jacobs, Kasten, Jr., Demott, & Wolfson: Laboratory Test Handbook, 2<sup>nd</sup> ED., Lexi-Comp Inc., 1990, pp. 709-710 and 759-760.
3. Baron, J.E. & Finegold, S: Baily and Scott's Diagnostic Microbiology, 8<sup>th</sup> ED., Mosby, 1990, pp. 226-270.
4. Lichtman R & Papera S: Gynecology (Well-Woman Care), Appleton & Lange, 1990, p. 45.

## Appendices

- Components of the Compound Microscope
- Microscope Preventive Maintenance
- Equipment Maintenance and Repair Log
- Diagnostic Criteria for Vaginal Wet Mounts
- Examples of Microscopic Images of Vaginal Wet Mounts

## COMPONENTS OF THE COMPOUND MICROSCOPE

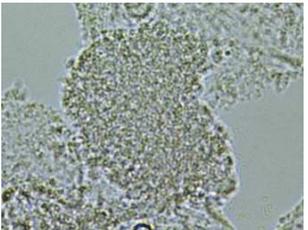


## MICROSCOPE PREVENTIVE MAINTENANCE

### Olympus, Swift, A-O Spencer, Bausch & Lomb

- Always read the specific instruction manual for the microscope before attempting to operate it. Make sure you know what all the controls do, and turn all knobs slowly.
- Document problems with the microscope on the Preventative Maintenance Log (see below).
- All microscopes should be used in the patient exam room or the designated laboratory area. The microscope should be placed on a flat, stable surface.
- Microscopes should never be located in any high traffic area, or where food is stored or consumed.
- When carrying the microscope, use two hands; one hand should support the bottom, and the other should have a firm grip on the arm. Be sure to carry it high enough so that it does not bump against anything.
- Lenses are very delicate and should not be touched directly; touching the lenses can cause smudges, possible scratches, or even a break. If lenses become smudged:
  - Lenses should be cleaned using special **lens tissue** designed for cleaning microscope lenses.
    - Use clean, dry lens tissue or lens tissue moisten with a **lens cleaner** designed for microscopes, followed by dry lens tissue to remove any residues from the lens. Do not apply force or rub vigorously since this can scratch lenses.
  - Never use soap, alcohols, xylene, or other solvents to clean lenses; these can cause build-up of moisture in the lens housing or destroy the cement holding the lens in place.
  - Never clean lenses with Kleenex or paper towels; these will scratch the lens.
- Cleaning:
  - The microscope stage and other surfaces should be cleaned periodically with a bactericidal disinfectant or mild soap and water to remove residues. If surfaces become visibly contaminated with body fluids, decontaminate with a bactericidal disinfectant then remove residues with water and dry. Be very conservative in the application of liquids to all surfaces since moisture could damage components.
  - **Be sure to clean and disinfect the eyepieces.** The eyes, nose, and mouth spread germs and bacteria very easily, and it's realistic someone could catch an infection from a dirty eyepiece. Clean lenses with lens tissue and lens cleaner and wipe other surfaces with a soft cloth and disinfectant glass cleaner after every use.
- Storage: when you have finished using the microscope:
  - Place the objective lenses on the lowest setting.
  - Use the coarse adjustment to put the nosepiece on its lowest setting.
  - Turn-off the light source - this will save energy, improve the longevity of the light, and prevent the light housing from getting too hot to the touch.
  - Store the microscope in a storage cabinet or cover the microscope with the dust cover.

## DIAGNOSTIC CRITERIA FOR VAGINAL WET MOUNTS

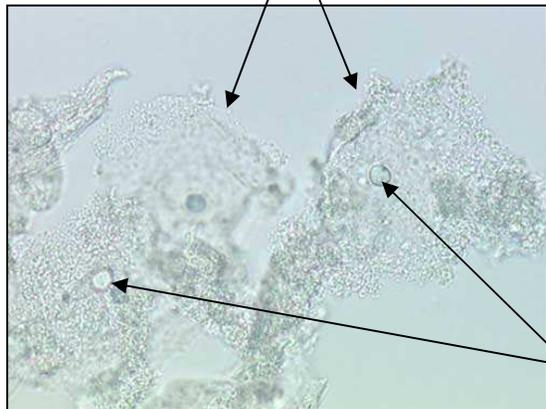
	<b>Normal</b>	<b>Bacterial Vaginosis</b>	<b><i>Trichomonas Vaginitis</i></b>	<b><i>Candida Vulvovaginitis</i></b>
<b>Vaginal pH</b>	<b>3.8-4.2</b>	<b>&gt;4.5</b>	<b>&gt;4.5</b>	<b>Usually ≤4.5</b>
<b>Discharge</b>	Clear to white, flocculent	Thin, homogeneous, milky white, adherent, often increased; malodorous, “foul and fishy”	Yellow-green or gray, frothy, increased; malodorous	White, thick and clumpy, 'cottage cheese-like', sometimes increased
<b>Amine odor (KOH) Whiff test</b>	Absent	Present (fishy)	Often present (fishy)	Absent
<b>Main patient complaints</b>	None	Milky discharge, bad odor (possibly worse after intercourse), possible itching	Frothy discharge, bad odor, vulvar pruritus, dysuria; 50% asymptomatic	Itching, burning, discomfort, dysuria, thick discharge
<b>Microscopic</b>				
	Abundant rod-shaped lactobacilli; epithelial cells; few or no WBCs	≥20% clue cells with adherent coccoid bacteria; no or few WBCs	Trichomonads; many WBCs (>10/hpf)	Budding yeast, pseudohyphae or spores if non-albicans species; usually few WBCs

## EXAMPLES OF MICROSCOPIC IMAGES OF VAGINAL WET MOUNTS

The definition of a **clue cell** requires  $\geq 75\%$  of the cellular edges of the squamous epithelial cell to be obscured by bacteria; however, occasionally the cell nucleus may be observed in a clue cell even if the cell margin is totally obscured (as in some of the cells in the photomicrographs).



**Clue cells**



**Nuclei**



**Clue cells**

**Nucleus**

The cell membranes of **normal epithelial cells** are smooth and well defined. Occasionally, the edge of these cells may appear folded.

**Normal epithelial cells**



**Sperm**

**Normal epithelial cells**



**Normal epithelial cells with folded edges**

