

**Diagnosing Vaginal Infections**

---

**with the  
Wet Prep Procedure**

presented by  
Christie A. Grueser, M.S.S. MT (ASCP)

---

---

---

---

---

---

---

---

**Importance of Establishing  
Correct Diagnosis**

---

**Don't treat patients who are not infected – reduce  
the emergence of resistant organisms**

Goal of proper diagnosis and treatment:  
Re-establish beneficial vaginal flora and healthy  
mucosal surfaces which may reduce the  
transmission of several genital pathogens  
including

- HIV
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*
- *Trichomonas vaginalis*

---

---

---

---

---

---

---

---

**Appearance of Normal Vaginal Discharge**

---

- Normal D/C: whitish, creamy vaginal content, with  
clear mucous at cervix.
- See squamous epithelial cells
- few WBC's (<10/hpf)
- pH @ 4.0 – inhibits development of BV and growth of  
Trichomonads

20-60% of those complaining of vaginal discharge do  
not have clinical vaginitis

---

---

---

---

---

---

---

---

## Normal Vaginal Flora

**Composed of:**  
*Gardnerella vaginalis* – found in 40-60% of healthy women  
*Staphylococcus epidermidis*, Enterococci,  
 group D Streptococcus, *Candida sp.*,  
 gram negative rods, Lactobacilli, *Mycoplasma hominis*

**Anaerobes**  
*Mobiluncus*, *Bacteroides*, *Peptostreptococcus* and  
 others

**Most important organisms are Lactobacilli**

---

---

---

---

---

---

---

---

## Role of Lactobacilli

- Predominant bacteria found in 95% normal vaginas
- Lactic Acid - interferes with "abnormal" bacteria
- H<sub>2</sub>O<sub>2</sub> - 100% in normal; 11% in BV (Eichenbach, 1933)  
 Acts to control microenvironment of the vagina by inhibiting  
 overgrowth of potentially pathogenic organisms including  
 yeast.
- HIV, *Trichomonas* and gonorrhoea infection risk  
 lower in women with H<sub>2</sub>O<sub>2</sub> producing strains

---

---

---

---

---

---

---

---

## Reduction in Number of Vaginal Lactobacilli

**Due to:**

- Use of antibiotics – may destroy Lactobacilli
- Action of a bacteriophage – sexually transmitted virus that infects and kills lactobacilli. May be transmitted in host bacterium or as a free virus.
- Douching
- Lack of estrogen

---

---

---

---

---

---

---

---

**Gram stain of vaginal secretions containing many Lactobacilli**  
 Gram positive (purple) rods




---

---

---

---

---

---

---

---

**Bacterial Vaginosis**

**Background:**

- Most common vaginal infection:
  - 10% in low risk populations
  - 64% in high risk populations
- Decrease in number of hydrogen peroxide producing Lactobacilli, resulting in overgrowth of anaerobes and *Gardnerella vaginalis*.

Organisms associated with BV produce mucolytic enzymes which permit their passage through the cervical mucous barrier into the upper genital tract.

---

---

---

---

---

---

---

---

**Bacterial Vaginosis - Causes**

- Decrease in number of hydrogen peroxide and lactic acid producing Lactobacilli resulting in an:
  - Increase in vaginal pH; usually greater than 4.5**
- Polymicrobial clinical syndrome due to an overgrowth of anaerobes, *Mycoplasma* and *Gardnerella vaginalis*
- Serve as a risk factor for premature labor, PID, postpartum endometritis and bacteremia; as well as HIV and other sexually transmitted diseases.
- 50% of all women with BV may be asymptomatic
- Cause – multiple sex partners, a new sex partner, douching or reduction in number of vaginal Lactobacilli for any reason.

---

---

---

---

---

---

---

---

## BV – Specimen Collection

**Specimen**

**Not recommended:**

- Culture - due to presence of *Gardnerella vaginalis* in low numbers as normal flora
- Pap smears

**Recommended:**

- Urethral and vaginal swabs of discharge material taken from posterior fornix, lateral vaginal wall as well as above and below the cervix.

---

---

---

---

---

---

---

---

## BV – Specimen Collection

**Procedure:**

Put vaginal rayon swabs into 2 test tubes labeled with the patient's name, date and time of collection:

**Tube #1 - 0.5cc warm saline – Clue Cells, yeast and Trichomonads**

**Tube #2 - 0.5cc warm 10% KOH – yeast and Whiff Test**  
**Note immediately if a fishy odor is emitted from the tube after the vaginal secretions are added to the KOH.**

---

---

---

---

---

---

---

---

## BV – Specimen Collection

2. Place small amount of the saline sample on a glass slide and coverslip
3. Examine on low power (10x) and high power (40x)  
Look for presence of Clue Cells, yeast and Trichomonads.
4. Place a small sample from KOH tube on glass slide and coverslip. Examine on low and high power for presence of budding yeast forms and/or pseudohyphae. (Not for proficiency testing. KOH will eventually destroy even yeast.)

---

---

---

---

---

---

---

---

## BV – Diagnosis Criteria

1. Malodorous milky (homogeneous) discharge.
2. Vaginal pH greater than 4.5
3. Positive Whiff Test
4. **20% of all squamous epithelial cells are "Clue Cells"**

---

---

---

---

---

---

---

---

## Clinical Diagnosis of BV

Clinical Criteria	Sensitivity	Specificity
Wet mount	High	High
Whiff test	Moderate	Moderate
pH test	High	Low
Discharge	low	Low

Presence of 3 or more of the above is indicative of BV

---

---

---

---

---

---

---

---

## Discharge Visualization

- Least sensitive and specific test for BV
- Quantitatively increased -- may appear as if milk was poured into the vagina
- Described as grayish-white and homogeneous
- Distinctive from discharge arising from yeast or *Trichomonas*

---

---

---

---

---

---

---

---

## Vaginal pH TEST

- pH indicator strips:** colorpHast pH 4.0-7.0  
Place sample of vaginal secretions on test strip, read while still moist
- pH > 4.5 indicates possible BV or *Trichomonas*
  - Be careful not to sample the cervix (Cervical secretions have a pH 7.0) - false pos.
  - Note: *Semen may have a buffering effect which may raise the vaginal pH for up to 3 hours thereby inhibiting growth of Lactobacilli leading to an overgrowth of BV associated organisms*

---

---

---

---

---

---

---

---

## KOH "Whiff Test"

- Procedure:**
- Swab containing vaginal secretions is placed in a test tube containing 0.5cc of 10% KOH. Immediately note if "fishy" odor is present
  - KOH volatilizes amines produced by anaerobic bacteria - results in a sharp "fishy" odor
  - Odor most noticeable following intercourse and during menstrual period.
- Microscopic exam:**
- Place sample from KOH tube on slide – examine on low and high power for presence of yeast pseudohyphae and budding forms.

---

---

---

---

---

---

---

---

## Wet Mount Preparation

- Procedure:**
- Insert swab into test tube containing 0.5cc of warm normal saline. Gently mix.
  - Place sample on glass slide and cover slip.
  - Visualize microscopically at both low (10x) and high power (40x). Scan for "Clue Cells" on low power and then confirm on high power.

---

---

---

---

---

---

---

---

## Microscopic Findings for BV

- Few or no white cells (<10/hpf) present because organisms do not invade the subepithelial tissue
- Absence of typical Lactobacilli-like rods
- **Presence of "Clue Cells"**  
**20% of the squamous epithelial cells need to be Clue Cells to call BV**

---

---

---

---

---

---

---

---

## Clue Cells

- Squamous epithelial cells, if gram stained, are covered with gram negative, gram positive and gram variable organisms.
- Borders are difficult to delineate.
- 75% of the border of the cell must be obscured by bacteria in order to call the cell a "Clue Cell"
- This attachment of organisms gives the cell a "furry" or stippled appearance. The cell appears to have been pressed in glitter or sand.

20% of all squamous epithelial cells must be Clue Cells for diagnosis of Bacterial Vaginosis

---

---

---

---

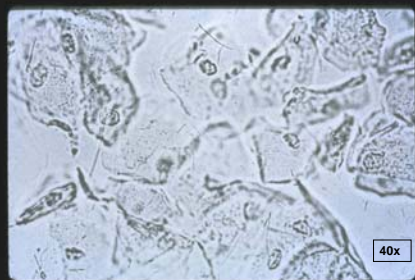
---

---

---

---

## Normal Squamous Epithelial Cells (seen in wet prep on high power)



---

---

---

---

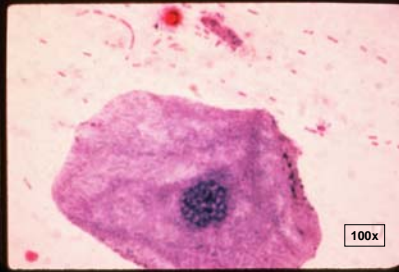
---

---

---

---

**Gram Stained Normal Squamous Epithelial Cell**



---

---

---

---

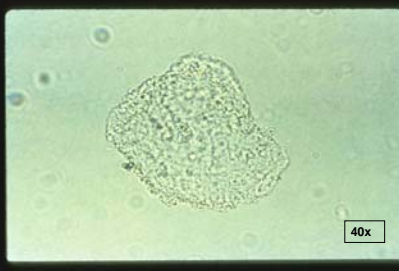
---

---

---

---

**Clue Cell**



---

---

---

---

---

---

---

---

**Normal Squamous Epithelial Cells and "Clue Cells"**



---

---

---

---

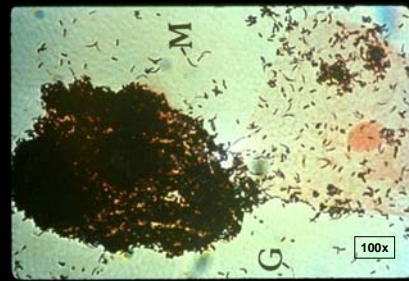
---

---

---

---

## Gram Stained Clue Cell and Normal Squamous Cell



---

---

---

---

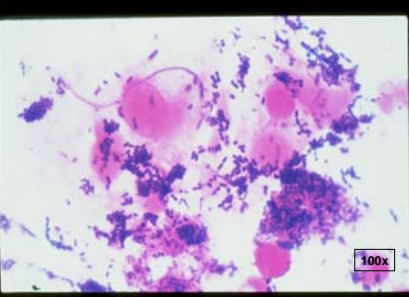
---

---

---

---

## Gram Stained Vaginal Secretions



---

---

---

---

---

---

---

---

## Bacterial Vaginosis Diagnosis

Presence of at least 3 of the following in a symptomatic woman without another cause of vaginal discharge:

1. Homogeneous white discharge
2. Vaginal pH > 4.5
3. Positive Whiff Test
4. Clue cells – (most sensitive finding)

---

---

---

---

---

---

---

---

### Additional Findings for BV

- 50% of all women with BV may be asymptomatic – usually not treated unless pregnant, presence of PID, mucopus and/or cervical tenderness is present

---

---

---

---

---

---

---

---

### Additional Diagnostic Techniques for BV

- Gram stain – Gold standard
- DNA probe-based tests
- Tests to detect pH and trimethylamines
- Tests to detect enzyme activity of *G. vaginalis* (prolineaminopeptidase)
- Tests to detect high levels of sialidase – enzyme produced by *G. vaginalis* and some anaerobes

---

---

---

---

---

---

---

---

### Treatment for Bacterial Vaginosis

**Nonpregnant Women:**

**Benefits:**

1. Relieve vaginal symptoms and signs of infection
2. Reduce risk of infectious complications following hysterectomy or abortion.
3. Reduce risk of STD transmission

---

---

---

---

---

---

---

---

## Treatment for Bacterial Vaginosis

**Nonpregnant Women:**

- Metronidazole gel (0.75%) - one 5 gm applicator intravaginally for 5 days
- Clindamycin phosphate cream 2% - one applicator intravaginally at bedtime for 7 days
- Metronidazole 500 mg orally bid for 7 days

**Alternative regimens:**

- Oral Clindamycin - 300 mg bid for 7 days
- Clindamycin ovules – 100 gm intravaginally for 3 days
- Single dose - 2gm oral Metronidazole – lowest efficacy*
- 750 mg extended release tables – 1 tablet/day for 7 days

PID – oral therapy for 14 days. CDC guidelines state a treatment regimen against Chlamydia and gonorrhoea must be prescribed.

---

---

---

---

---

---

---

---

## Treatment of Bacterial Vaginosis in Symptomatic Pregnant Patient

**Pregnant Women with low-risk pregnancy:**

**Benefits reduce**

1. risk of postpartum PID
2. adverse pregnancy outcomes
3. risk of preterm birth

---

---

---

---

---

---

---

---

## Treatment of Bacterial Vaginosis in Symptomatic Pregnant Patient

**Pregnant Women with low-risk pregnancy:**

- Metronidazole 250mg orally tid for 7 days
- Clindamycin 300 mg orally bid for 7days

*Topical metronidazole and clindamycin not recommended during pregnancy; may lead to prematurity and neonatal infections (particularly after using clindamycin cream)*

- Women at risk for preterm birth should be screened and treated for BV at first prenatal visit. Follow-up evaluation 1 month after completion of treatment.

---

---

---

---

---

---

---

---

## Recurrent BV Infection

**BV recurs within one month in about 30% of women**

**Causes:**

- Persistence of pathogenic bacteria
- Unidentified host factors
- Lactobacilli not re-colonizing in vagina
- Reinfection from male sexual partner?

Likelihood of relapse not affected by treating partner.

---

---

---

---

---

---

---

---

## Recurrent BV Infection

**Management:**

- Test of cure 2 - 3 weeks after therapy
- Intravaginal metronidazole twice weekly for 6 mos.
- Vaginal moisturizer prior and following intercourse
- Minimize semen exposure with use of condoms
- Test and treat sexual partners?
- Discourage use of exogenous lactobacilli
- Vulvovaginal care. Avoid douching, tampons and steroid creams

---

---

---

---

---

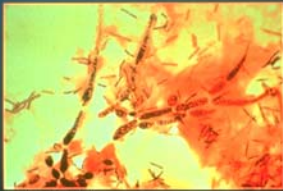
---

---

---

## Vulvovaginal Candidiasis

*Candidiasis*



Michael H. Hoffmann, MD

---

---

---

---

---

---

---

---

## Vulvovaginal Candidiasis

**Background:**

- Infections commonly occur as a result of long term administration of antibiotics which leads to a decrease in normal vaginal flora and the overgrowth of yeast.
- Steroids, immunosuppressants, antineoplastic drugs, and the prolonged use of indwelling catheters promote growth of yeast.
- Increased incidence with diabetic and pregnant patients
- Yeast present as normal flora in low numbers in vagina
- Majority of infections due to *Candida albicans*

---

---

---

---

---

---

---

---

## Vulvovaginal Candidiasis

**Clinical signs and symptoms:**

- Vaginal pH may be less than 4.5
- Thick cottage cheese-like discharge
- Whiff test negative
- Irritant vaginitis with urethritis and dysuria in some patients

---

---

---

---

---

---

---

---

## KOH Prep

Used only for detecting presence of yeast and the "Whiff Test"

**Principle:**

- Destroys cellular (protein) material to expose presence of yeast forms if present. Destroys white and red cells, squamous epithelial cells, Clue Cells and Trichomonads

**KOH Reagent:**

- Use warm which will accelerate the process of lysing cellular components

**Preps not permanent** – reagent will eventually dissolve yeast cells

---

---

---

---

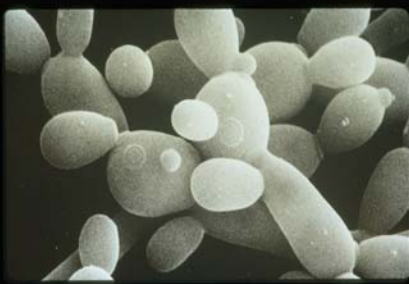
---

---

---

---

**Budding Yeast  
Electron Microscopy**



---

---

---

---

---

---

---

---

**Yeast  
Phase Contrast Microscopy**



---

---

---

---

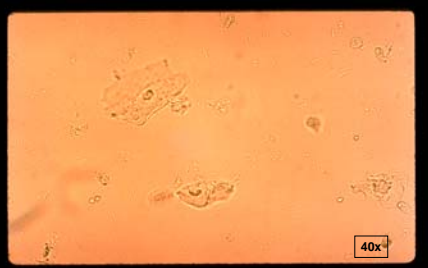
---

---

---

---

**Normal Vaginal Wet Prep**



---

---

---

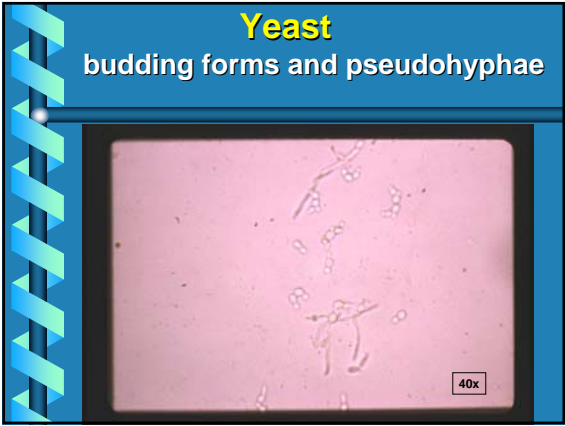
---

---

---

---

---



---

---

---

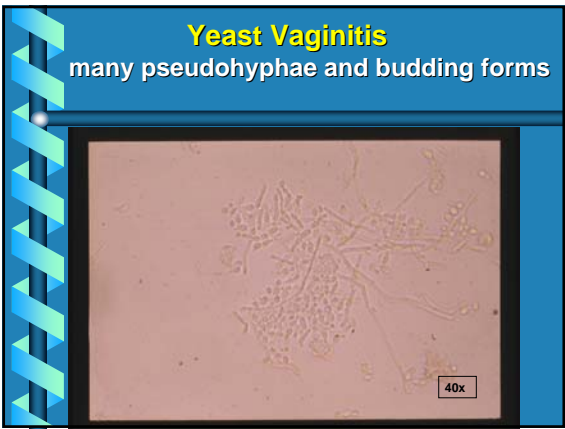
---

---

---

---

---



---

---

---

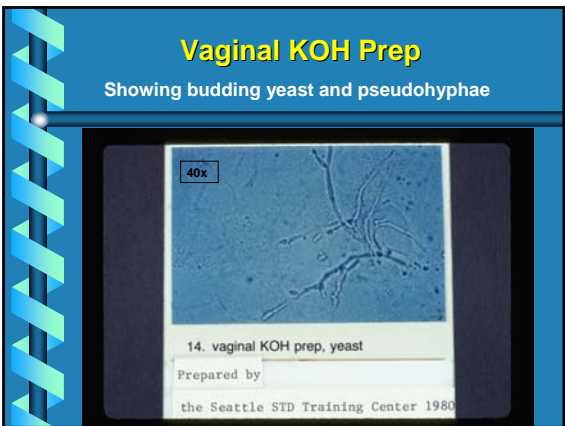
---

---

---

---

---



---

---

---

---

---

---

---

---

## Yeast – Gram Stain




---

---

---

---

---

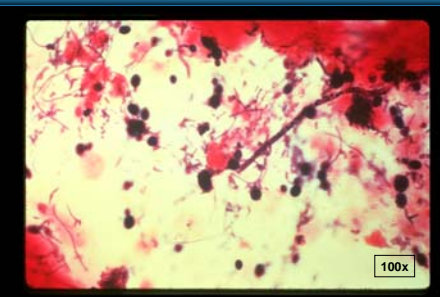
---

---

---

## Vaginal Prep

Gram stain showing budding yeast and pseudohyphae




---

---

---

---

---

---

---

---

## Yeast Vaginitis - Treatment

<p><b><u>Uncomplicated</u></b></p> <p>infrequent mild to moderate <i>Candida albicans</i> Not immunosuppressed</p>	<p><b><u>Complicated</u></b></p> <p>recurrent- &gt; 4 /year severe non-<i>albicans</i> pregnancy, diabetes immunosuppressed</p>
--	---

---

---

---

---

---

---

---

---

### Yeast Vaginitis - Treatment

- Topical 2% miconazole – 7 days  
*Use only topical treatment for pregnant patient*
- **Fluconazole** – oral – 150mg single dose
- **Follow up** – Return visits only if symptoms persist or recur within 2 months of onset of initial symptoms

---

---

---

---

---

---

---

---

### Yeast Vaginitis - Treatment

Sexual Partner

- Not treated
- Does not affect frequency of recurrence
- If balanitis exists, may benefit from topical antifungal treatment

---

---

---

---

---

---

---

---

### Yeast Vaginitis - Treatment

**Complicated:**

**Recurrent infections: > 4 episodes / year**

- Culture to confirm diagnosis
- if non-*albicans*: 7 - 14 days of non-fluconazole – requires longer duration of treatment
- To prevent relapse: 100mg/wk for 6 mos. and 600 mg boric acid capsules -1/day for 2 weeks
- non-*albicans* - longer duration of treatment
- Maintenance regimen of topical Nystatin – 7-14 days
- May see azole resistant *C. albicans* – need surveillance of recurrent isolates

---

---

---

---

---

---

---

---

## Yeast Vaginitis - Treatment

**HIV Patients:**

- Rate of infection correlates with severity of immunodeficiency
- With azole exposure – see more infections with non-*albicans* species
- Therapy same as for seronegative women
- Recurrent infection not an indication for HIV testing

---

---

---

---

---

---

---

---

## Trichomoniasis

---

---

---

---

---

---

---

---

## Trichomoniasis

**Background:**

- Transmitted via sexual contact
- Males act as reservoirs
- Overt infection in females
- Long duration of infection: 4 months in men and 5 years in women
- Does not survive in acid secretions of healthy adults – prefers alkaline pH
- Most common STD in African-American women
- 50%-70% of patients have sub-clinical infection
- Pregnant patient: related to premature rupture of membranes, preterm delivery and low birth weight
- May produce pneumonia in newborn after passing through infected birth canal

---

---

---

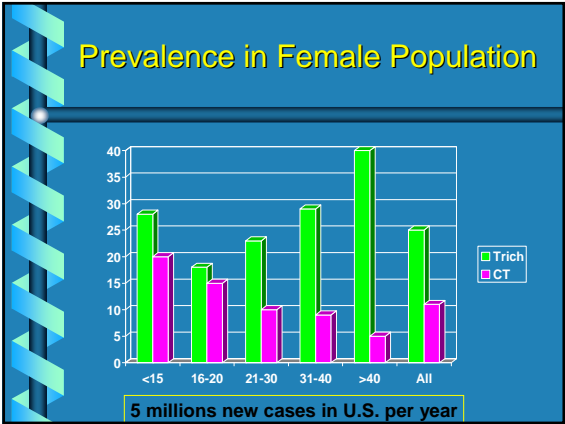
---

---

---

---

---




---

---

---

---

---

---

---

---

---

---

### Trichomoniasis Clinical Signs & Symptoms

Signs / Symptoms	% Women
Asymptomatic	50
Vulvar Erythema	40
Yellow Discharge	42
Frothy Discharge	8
Vulvar Itching	20-40
Strawberry Cervix	2

---

---

---

---

---

---

---

---

---

---

- ### Trichomoniasis
- Vaginal itching, inflammation and burning
  - Frothy brown malodorous discharge containing bacteria, white cells, epithelial cells and motile trichomonads
  - Vaginal pH > 4.5 – due to decrease in hydrogen peroxide producing lactobacilli

---

---

---

---

---

---

---

---

---

---

## Transmissison

- Sexual contact
- Incubation period: 4-28 days

---

---

---

---

---

---

---

---

## Trichomoniasis

**Specimens**

- Vaginal secretions
- Spun urines
- Urethral discharges
- Prostatic massage
- Pap smears??

**DO NOT REFRIGERATE SPECIMEN – WILL KILL TRICHOMONADS**

---

---

---

---

---

---

---

---

## Wet Prep Procedure

**SPECIMEN**

- A. Vaginal discharge collected on a cotton or rayon swab and placed into a tube with sterile saline solution (0.5 – 1ml.). Label specimen tube with a barcode label, (if available)
- B. Urethral discharge collected on a cotton or rayon swab and placed into a tube with sterile saline solution (0.5 – 1ml.). Label specimen tube with a barcode label.
- C. **Specimen rejection criteria:**
  1. Unlabeled or improperly labeled specimens
  2. Specimens received in leaking, cracked or broken containers
  3. Specimens received dry without saline solution or with more than 1 ml. saline solution.
  4. Specimens received more than one hour after collection

---

---

---

---

---

---

---

---

## Wet Prep Procedure

**MATERIALS**

**Reagents**  
Normal saline

**Supplies**  
Disposable plastic pipettes  
Microscope slides (1 by 3 in. or larger)  
22 x 22mm coverslips

**QUALITY CONTROL**

- The saline should be clear with no visible contamination.
- Calibration of the microscope has occurred within the last 12 months.

**NOTE: THIS IS CONSIDERED A STAT PROCEDURE. EXAMINE WITHIN 30 MINUTES OF RECEIPT OF THE SPECIMEN**

---

---

---

---

---

---

---

---

## Wet Prep Procedure

A. Transfer 1 drop of saline suspension to the microscope slide.  
B. Cover the specimen with the coverslip.  
C. Examine with the low power (10x) objective first.  
D. Examine the entire coverslip for motile *Trichomonads*. Suspicious objects may be examined with the high power (40x) objective.  
    - The organism displays directional, jerky motility. It is slightly larger than a PMN.  
E. Using the high power (40x) objective, examine the suspension for trichomonads and PMN's.

PMN's (polymorphonuclear cells) note if >10/hpf  
PMN's are seen in *Trichomonas* vaginitis, *Chlamydia*, herpes, GC infections

---

---

---

---

---

---

---

---

## *Trichomonas vaginalis*

**Morphology (warm saline prep)**

- Pear-shaped
- Motile – with jerky or “spinning” motility
- Will lose motility, round up and resemble a wbc
- 3-5 anterior flagella
- Oval and tapers to a point at posterior end
- **Organism cannot be accurately identified in saline mount if it has lost its motility**
- Specimen must be examined immediately – organism dies quickly
- Chronic infection – see fewer organisms
- Organism feeds on wbc's and bacteria

---

---

---

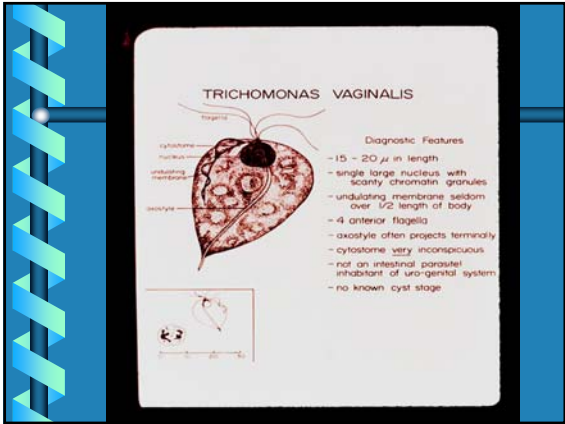
---

---

---

---

---




---

---

---

---

---

---

---

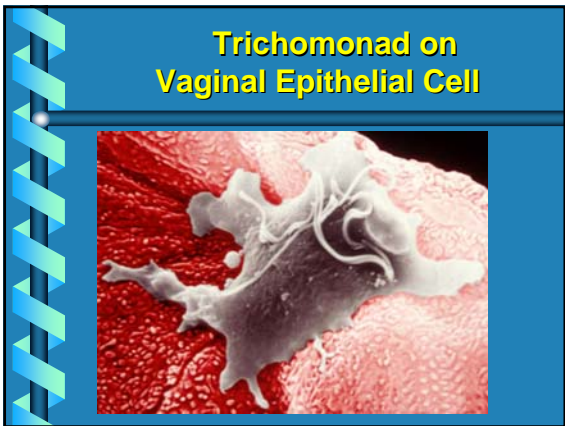
---

---

---

---

---




---

---

---

---

---

---

---

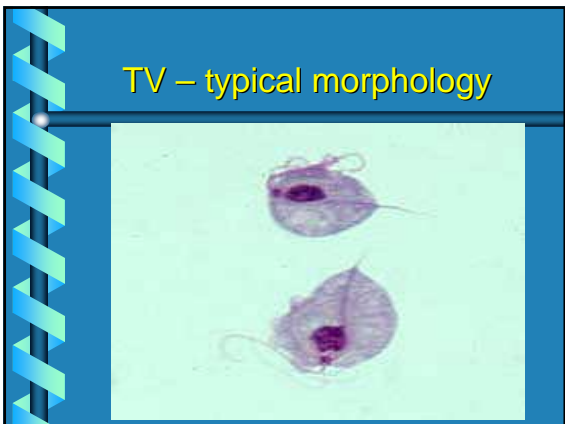
---

---

---

---

---




---

---

---

---

---

---

---

---

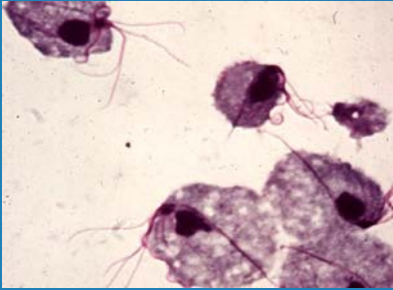
---

---

---

---

*Trichomonas vaginalis*



---

---

---

---

---

---

---

---

Wet Prep Appearance (40x)



---

---

---

---

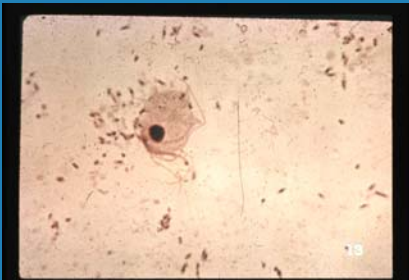
---

---

---

---

*Trichomonas hominis*



---

---

---

---

---

---

---

---

## Wet Prep Procedure Reporting

1. **Trichomonas**
  - Positive
  - Negative
2. **PMN's**
  - Normal: if = <10 PMN's/hpf
  - Increased: if = >10 PMN's/hpf

**PROCEDURE NOTES**  
It is very important that specimens be examined within one hour after collection. After one hour, Trichomonads lose their motility.

**LIMITATIONS**  
Wet preps have been reported to detect *T. vaginalis* in 52-75% of infected patients. Other alternative diagnostic methods include culture, monoclonal antigen detection kits and permanent stained smears.

---

---

---

---

---

---

---

---

## Trichomoniasis: Other Diagnostic Tests

- Saline suspension - 52 – 75% sensitivity
- pH >4.5
- Positive Whiff test
- Stained Slide prep (Pap Smear)
- Culture – **Gold Standard**
- PCR 84% sensitivity – currently research only
- Trichomonas Rapid Test – antigen detection
- Nucleic acid probe tests for *T. vaginalis*, *Candida albicans*, and *Gardnerella vaginalis*

---

---

---

---

---

---

---

---

## Diagnosis of *T. vaginalis* in Females PCR vs. Culture vs. Wet Mount

Method	Sensitivity (%)
Wet Mount	52%
Culture	78%
PCR	84%

Legend: n= 337 women

---

---

---

---

---

---

---

---

## Trichomoniasis

**Treatment:**

- Single Dose Therapy – 2gm oral **metronidazole**  
(Dosage used for pregnant patients after 37<sup>th</sup> week – discontinue before breast feeding)
- 2gm **Tinidazole** (Category C)– not for pregnant patients
- Alternate regimen - 500mg metronidazole bid for 7 days
- Do not treat with metronidazole gel

**Treat sexual partner:**  
Treat with metronidazole – 2 gm oral single dose  
Avoid intercourse until therapy completed and both patient and partner are asymptomatic.

---

---

---

---

---

---

---

---

## Trichomoniasis Treatment failures

**Re-treat with:**

- 500mg metronidazole bid for 7 days
- 2 gm tinidazole (single dose)
- 2 gm per day for 5 days of either metronidazole or tinidazole

**Continued failure:**

- Consult infectious disease specialist
- Send organism for metronidazole susceptibility testing to CDC

---

---

---

---

---

---

---

---

## Trichomoniasis Treatment of Sexual Partner

- Treat with metronidazole or tinidazole – 2gm single dose
- Avoid intercourse until therapy is completed and both patient and partner are asymptomatic

---

---

---

---

---

---

---

---

## Trichomoniasis

**Association with HIV**

- Cofactor in amplifying HIV transmission
- May act to expand the portal of entry for HIV in an HIV negative person
- Pathology of trichomoniasis can increase HIV shedding

---

---

---

---

---

---

---

---

## Trichomoniasis

**Pathology**

- Induces mucosal hemorrhages – allows for direct viral access to bloodstream
- Elicits local cellular immune response with inflammation of the vaginal epithelium. Induces infiltration of white cells including CD4 cells and macrophages = increased number of HIV target cells.
- *Trichomonas vaginalis* degrades secretory leukocyte protease inhibitor thereby increasing HIV cell attachment.
- In HIV + patient – increase level of virus in body fluids

---

---

---

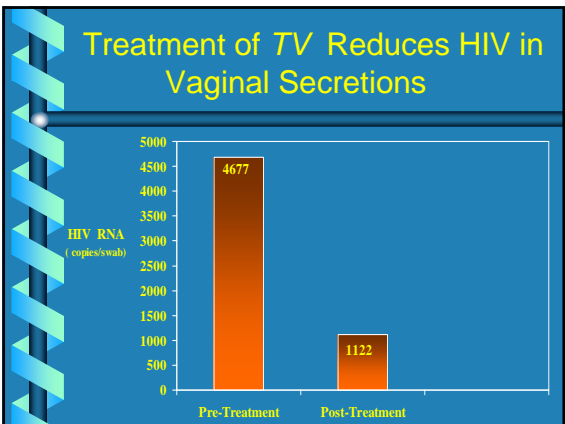
---

---

---

---

---




---

---

---

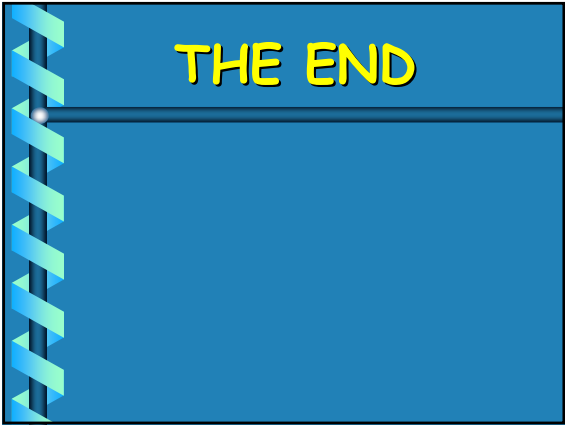
---

---

---

---

---



---

---

---

---

---

---

---