

Microscopy Competency/Training For Clinic-Based Providers

OSBHCN

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Learning Objectives

Following completion of the presentation, the participant will be able to:

- **Understand the CLIA PPM Laboratory Regulations**
- **Review Basic Microscope Use**
- **Review Vaginal Wet Preparation Procedure**
- **Identify organisms, cells and other elements found in a vaginal wet preparation**
- **Acquire Documentation for Annual CLIA PPM Competency**

CLIA

Clinical Laboratory Improvement Act

CLIA defines labs as:

- **Non-Waived (High Complexity and Moderate Complexity, including PPM)**
- **Waived**

Provider Performed Microscopy



A subcategory of the Moderate Complexity level was added after the original CLIA publication. It was first described as Physician Performed Microscopy and is now called Provider Performed Microscopy (PPM). The criteria for classifying these tests include:

- The examination must be performed by a physician or a mid-level practitioner (i.e., nurse practitioners, nurse midwives and physician assistants) during the patient visit on a specimen obtained from the provider's patient or a patient of the group practice.
- The procedure must be in the moderately complex category.
- The primary instrument for the test must be a microscope.
- The specimen must be labile or a delay in testing could compromise the accuracy of the test.
- Control materials are not available to monitor the entire testing process.
- Limited specimen handling is required.

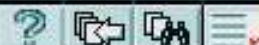
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Provider Performed Microscopy

Tests included in the PPM category are:

- Direct wet mount preparations for the presence or absence of bacteria, fungi, parasites and humancellular elements
- All potassium hydroxide (KOH) preparations
- Pinworm examinations
- Fern tests
- Post-coital direct, qualitative examinations of vaginal or cervical mucus
- Urine sediment examinations
- Nasal smears for granulocytes
- Fecal leukocyte examinations
- Qualitative semen analysis (limited to the presence or absence of sperm and detection of motility)

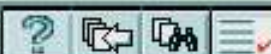
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CLIA January 2003

PPM Guideline Revisions

- **Proficiency Testing**
- **Facility Administration for Non-Waived tests**
- **Quality Systems for Non-Waived Tests**
- **Moderate & High Complexity referred to as Non-Waived testing**
- **Regulations parallel flow of patient specimen**

Provider Performed Microscopy



Proficiency Testing

Because the PPM tests are considered "non-regulated", proficiency testing is not specifically required but a laboratory is responsible for documenting quality assessment. The laboratory has two options in fulfilling this requirement: proficiency testing or split sampling. Most proficiency testing providers have specimens available for non-regulated analytes (usually one or two specimens per event instead of five, resulting in a lower cost). The second option is:

Split sampling is a comparison technique used to demonstrate accuracy in laboratory testing. It involves either collecting two specimens at the same time or dividing a single specimen in half. The test is then performed by both the laboratory initiating the testing and a second laboratory, such as a hospital or reference laboratory. Offices with multiple providers may utilize this technique to document the accuracy of provider performed microscopy procedures. In order to meet CLIA requirements for documentation of accuracy, split sampling must be done a minimum of two times per year.

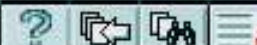
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Competence Assessment

All providers performing microscopic examinations should be assessed periodically (once upon hire, six months after hire, and annually thereafter)

Please refer to Competency Brochure

Provider Performed Microcopy (PPM)

Personnel Requirements:

- The CLIA personnel requirements are found in Subpart M of the Code of Federal Regulations (CFR) on CDC website
- The Laboratory Director must meet one of the following requirements: Is a Physician, Is a midlevel practitioner (midwife, nurse practitioner, or physician assistant, authorized by a State to practice independently in the State in which the laboratory is located); or is a Dentist.

Training/Credentialing

- **Persons performing PPM procedures must have documentation of educational qualifications and training before testing patient specimens.**
- **It is helpful to use a training checklist, which covers all essential functions of the job**

Provider Performed Microscopy



Quality Systems for Nonwaived Testing

Laboratories that perform non-waived testing (which includes PPM) are required to establish and maintain written policies and procedures that implement and monitor quality systems for all phases of the total testing process (preanalytic, analytic and post analytic) along with general laboratory systems.

General laboratory system requirements include monitoring and evaluating the overall quality of the lab and correcting identified problems. Further, the lab must:

- Ensure confidentiality of patient information.
- Establish and follow written policies and procedures that ensure positive identification and optimum integrity of a patient's specimen from the time of collection through completion of testing and reporting of results.
- Have a system to document and investigate all complaints and problems reported to the laboratory.

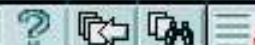
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Provider Performed Microscopy



Quality Systems for Nonwaived Testing

- Having test requisitions that include a request for the following information: the name and address of the authorized person ordering the test; the patient's name or unique identifier; the patient's sex and age or date of birth; the tests to be performed; the specimen source; the date and, if appropriate, time of collection; and, any additional information relevant and necessary for a specific test to ensure accurate and timely reporting of results.
- Establishing and following written procedures for patient preparation, specimen collection, labeling (patient name or unique identifier, source), storage and processing. Specimen acceptability and rejection requirements must also be documented.
- Establishing and following written policies and procedures for an ongoing mechanism to monitor, assess and correct problems identified in the preanalytic phase.

Requirements for the analytic phase of testing include:

- Having a written procedure manual with instructions for all three testing phases.
- Testing performed following the manufacturer's instructions.

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Provider Performed Microscopy



Inspections

The CLIA inspection regulations are found in Subpart Q of the Code of Federal Regulations; this subpart addresses both basic and specific inspection requirements. All laboratories issued a CLIA certificate and all CLIA-exempt laboratories must comply with the applicable inspection requirements. CMS's policy for inspections includes announced initial and recertification inspections and unannounced complaint, and follow-up inspections. The process focuses more on outcomes as opposed to processes. While laboratories issued a certificate for provider performed microscopy are not subject to biennial inspections, CMS or its representative may conduct inspections at any time during the laboratory's hours of operation to:

- Determine if the laboratory is operated and testing is performed in a manner that does not constitute an imminent and serious risk to public health.
- Evaluate a complaint from the public.
- Determine whether the laboratory is performing tests beyond the scope of the laboratory's certificate.

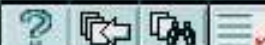
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General Microscope Information



Normal Vaginal Wet Mount

Normal Vaginal pH: 3.8 - 4.2

KOH prep (normal):

- All cells are lysed
- Yeast buds and/or Pseudohyphae not present

Amine odor (Whiff test) normal:

- negative

Saline prep (normal):

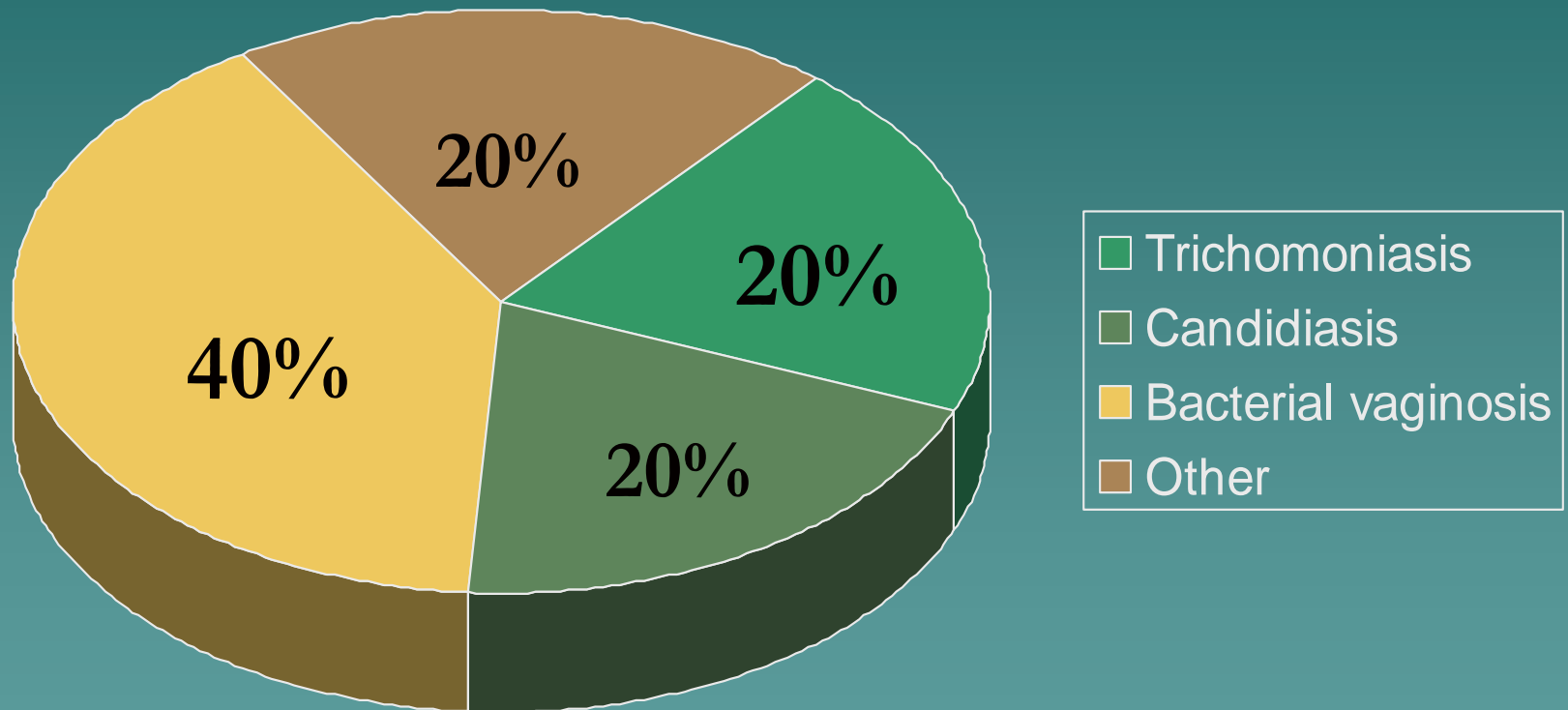
- Normal squamous epithelium
- Lactobacillus present
- Trichomonads not present
- Yeast buds and/or Pseudohyphae not present

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
- Lactobacilli are most important organism to keep healthy vaginal environment

Vaginitis: Etiologies



“Other” can include mixed infections including herpes, chlamydia, atrophic, irritant/chemical, desquamative interstitial vaginitis; erosive lichen planus

Vaginitis: Differentiating BV, Candidiasis, and Trichomoniasis

	NORMAL	BACTERIAL VAGINOSIS	CANDIDIASIS	TRICHOMONIASIS
Symptoms presentation		Odor, discharge, itch	Itch, discomfort, dysuria, thick discharge	Itch, discharge, asymptomatic
Vaginal discharge	Clear to white	Homogenous, adherent, thin, milky white; malodorous “foul fishy”	Thick, clumpy, white “cottage cheese”	Frothy, gray or yellow-green; malodorous
Clinical findings			Inflammation and erythema	Cervical petechiae “strawberry cervix”
Vaginal pH	3.8 - 4.2	> 4.5	Usually ≤ 4.5	> 5
KOH “whiff” test	Negative	Positive	Negative	Often Positive
NaCl wet mount	Lacto-bacilli	Clue cells ($\geq 20\%$), no/few WBCs	Few to many WBCs	Motile flagellated protozoa, many WBCs
KOH wet mount			Pseudohyphae or spores if non- <i>albicans</i> species	

Examination for detecting vaginitis

Pelvic Exam

- ◆ Allows visual examination of vaginal cavity and cervix and collection of vaginal secretions for analysis

Wet Mount

- ◆ Involves examination of vaginal secretions under the microscope. A wet mount should be performed in all symptomatic patients and in asymptomatic patients when abnormal discharge is detected

pH Test

- ◆ Indicates which infection occurs at what PH scale

KOH Whiff Test

- ◆ A mix of 10 – 20% KOH and vaginal fluid helps detect a foul, fishy, unpleasant odor. All cells except fungus are cleared

Specimen Collection for Wet Mount

- Proper specimen collection, labeling, and handling are essential for good results (GIGO)
- Collect specimen by swabbing lateral vaginal walls and vaginal pool to collect epithelial cells and secretions
- Avoid contact with cervical discharge

Specimen Preparation for Wet Mount

- Place the swab in small labeled test tube containing 0.5ml RT or warm saline (0.9% NaCl)
- Vigorously rotate the swab in the saline
- Use the same swab to place two specimens onto two labeled glass slides (One for Saline & one for KOH)

pH PAPER (narrow range best)



pH Test

Press pH paper against vaginal wall or smear vaginal discharge onto the pH paper with a swab
– careful not to sample the cervix!

Match the resulting color to the pH color scale

Normal Vaginal pH 3.8 – 4.2. Lactobacilli produce lactic acid, which aids in keeping healthy vaginal environment. Some Lactobacilli produce H_2O_2 (weak acid and microbicide)

Factors falsely affecting pH

- Saline elevates pH
- Cervical and menstrual secretions have higher pH
- Lubricants and Douching

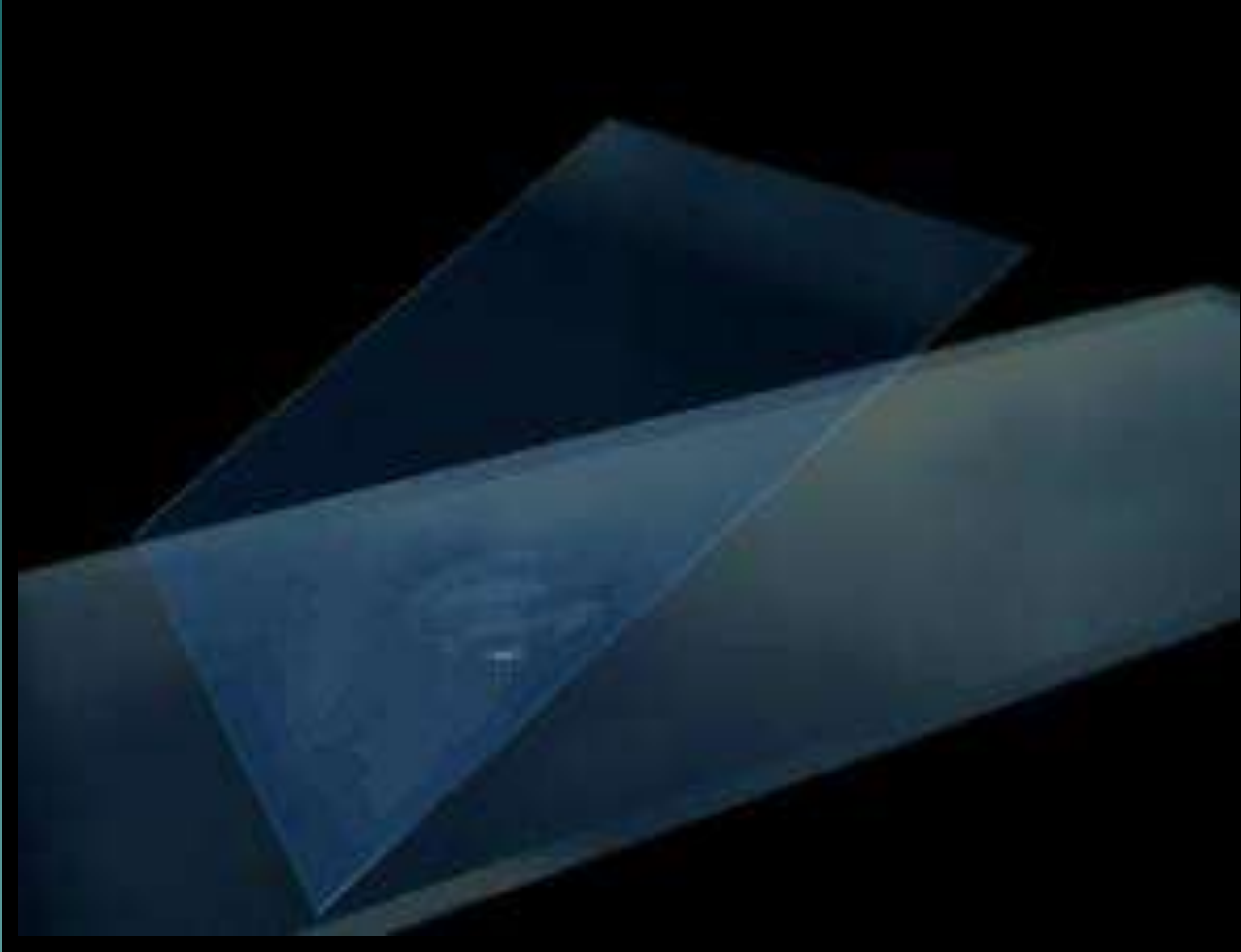
Note: *Semen may have a buffering effect which may raise the vaginal pH for up to 8 hours thereby inhibiting growth of Lactobacilli leading to an overgrowth of BV associated organisms*

Amine Odor (Whiff) test

Potassium hydroxide (KOH)

- Add warm 10% KOH to one vaginal specimen on slide
- Sniff immediately for fishy odor
- Odor most noticeable following intercourse and during menstrual period.
- Add warm Saline (0.9% NaCl) to the second vaginal specimen
- Then add a cover-slip to each specimen
- Proceed with microscopic evaluation

Apply coverslip in one direction →



- ◆ The coverslip is the optical window through which you view the specimen.
- ◆ Most specimens are placed on a glass slide and covered with a thin glass coverslip.
- ◆ Coverslip quality, thickness, and cleanliness is critical for good microscopy

Microscopic Evaluation

- **Start under low power (10x) objective**
- **Scan the saline sample first for presence of**
 - WBCs
 - Motile trichomonas
 - Yeast buds or pseudohyphae
- **Scan the KOH sample second for:**
 - Yeast buds or pseudohyphae
 - Other cells are lysed

Microscopic Evaluation – cont.

**Under high power (40x)
objective**

Analyze saline sample for

- Clue Cells
- WBCs
- Lactobacillus
- Motile trichomonas
- Yeast buds or pseudohyphae

Analyze KOH sample further

- Yeast buds or pseudohyphae
- Other Cells are lysed

Reading KOH Slide

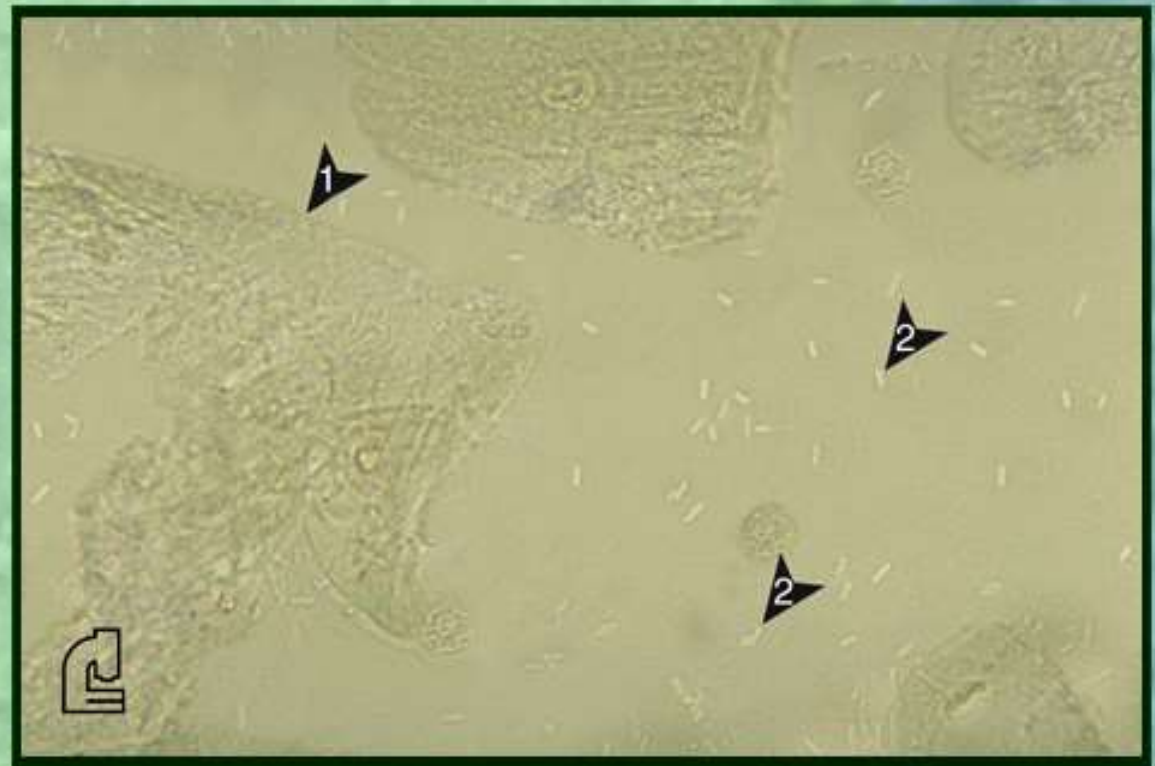
- KOH destroys cellular protein (RBC, RBC, and Squamous cells). Identifies presence of Yeast
- KOH is alkaline and will volatilize the amines. Can QC using pH paper and expiration date
- Read for Yeast on low and high power

Vaginal Wet Preparation - Cells



Squamous epithelial cell

The #1 arrowed object in this photograph is a squamous epithelial cell. The #2 arrowed objects are bacteria.



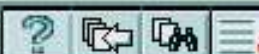
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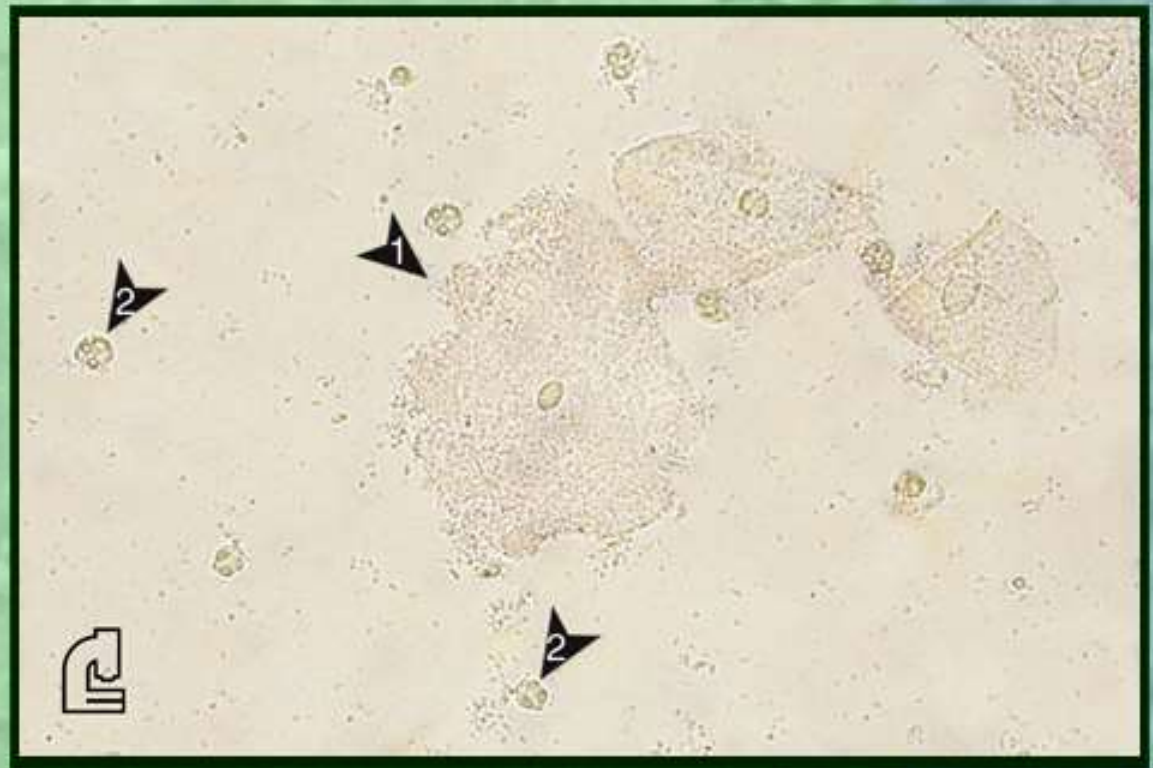
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Vaginal Wet Preparation - Cells



Clue cell

The #1 arrowed object in this photograph is a clue cell. The #2 arrowed objects are white blood cells.



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Vaginal Wet Preparation - Cells



Clue cell cont.

The #1 arrowed object in this photograph is a clue cell. The #2 arrowed object is a squamous epithelial cell.



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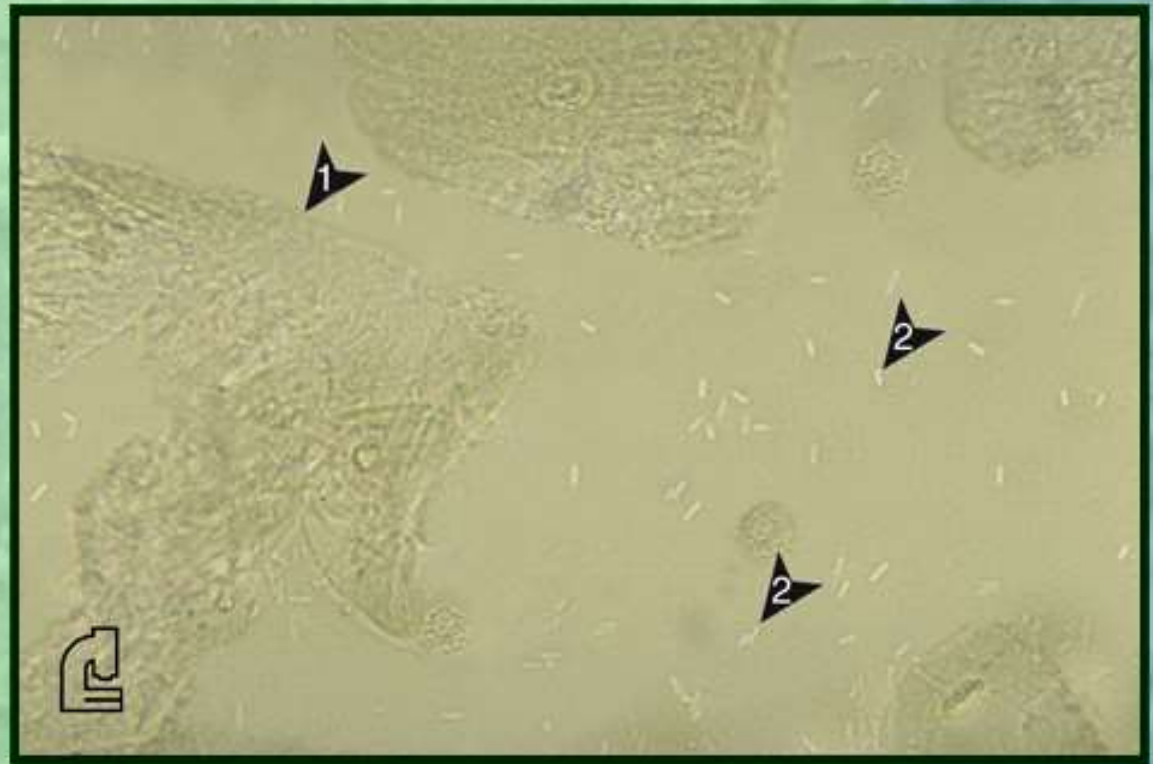
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Vaginal Wet Preparation - Organisms



Bacteria

The #1 arrowed object in this photograph is a squamous epithelial cell. The #2 arrowed objects are bacteria.



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Quantitation (Gradation) Required

Rare	Less than 10 organisms or cells/slide
1+	Less than 1 organism or cell/HPF
2+	1 to 5 organisms or cells per HPF
3+	6-30 organisms or cells per HPF
4+	Greater than 30 organisms or cells/ HPF

Diagnosis of Bacterial Vaginosis (BV)

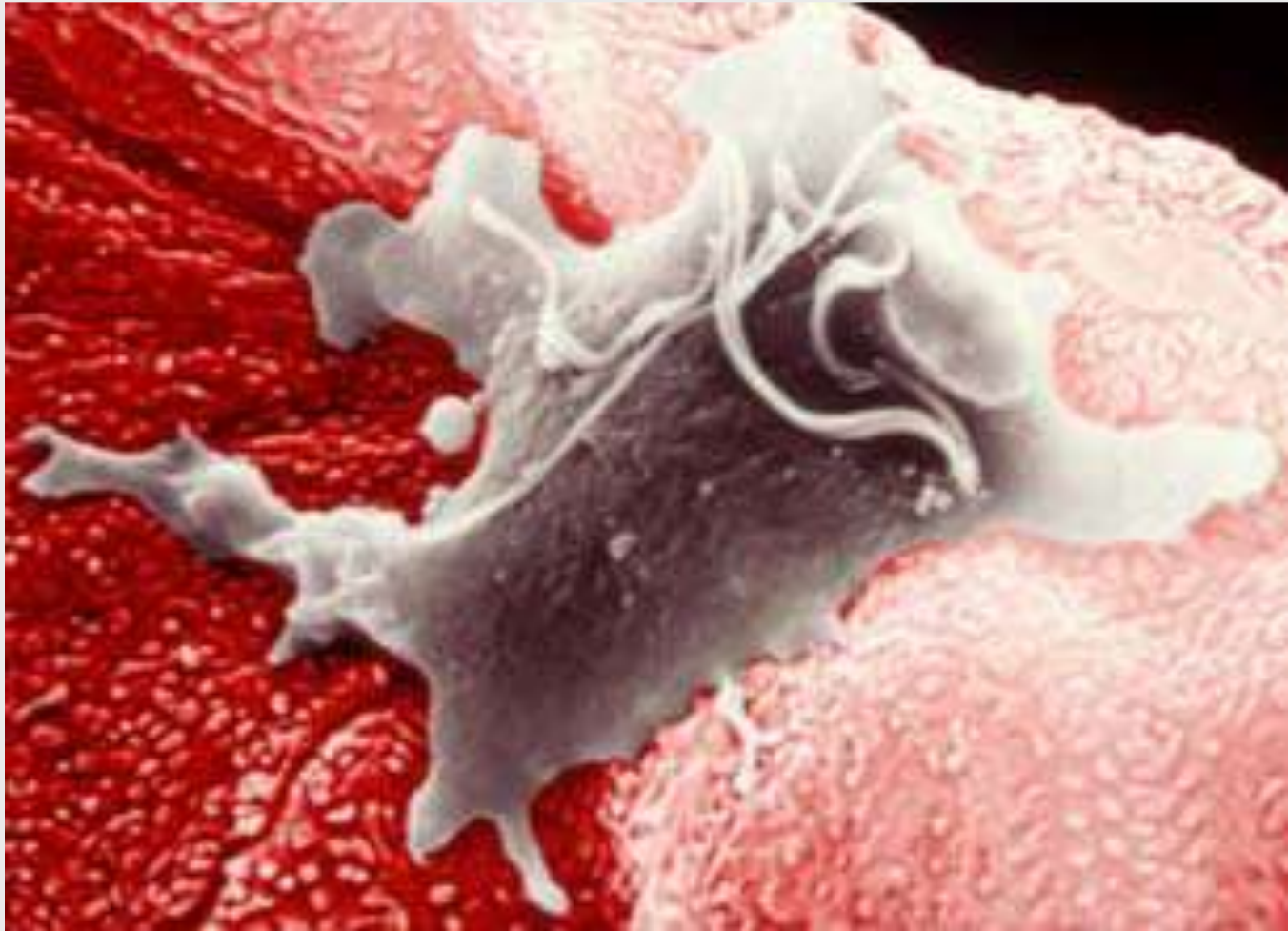
- BV is referred to as a 'vaginosis' rather than an 'vaginitis' because 'itis' implies an inflammation which would be accompanied by a proliferation of white blood cells (WBC's)
- In BV the bacteria flourish without a significant increase in WBC's (Leukocytosis)
- Lactobacilli decreased or absent
- Presence of “Clue Cells”

Bacterial Vaginosis

Clinical diagnosis is defined by 3 of the 4 criteria:

1. pH > 4.5
2. 20% of the squamous epithelial cells need to be Clue Cells on microscopic evaluation
3. Positive KOH “Whiff” test
4. Presence of homogeneous, non-viscous, milky white vaginal discharge

Trichomonad on Vaginal Epithelial Cell



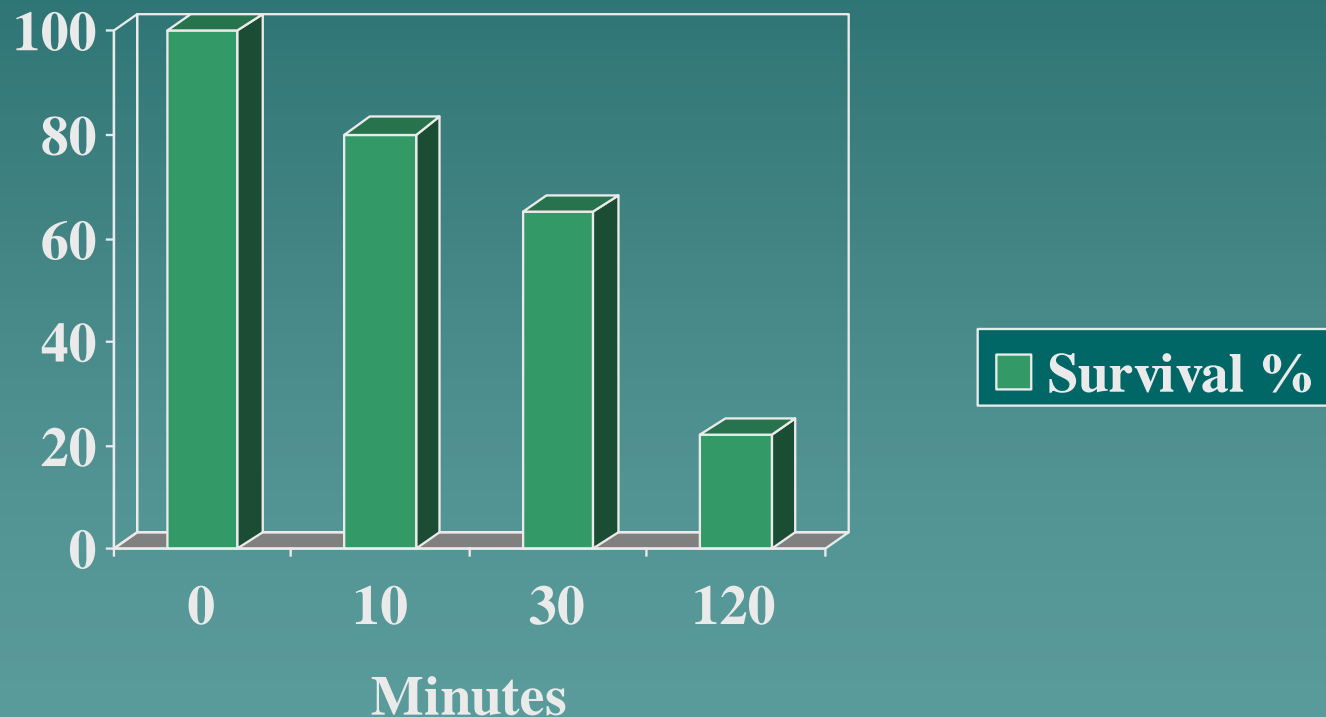
Bacterial Vaginosis

Other BV Diagnostic tests:

- Gram Stain – Gold Standard
- DNA Probe test kits (better in laboratory)
- Testing for Proline Aminopeptidase Activity
- Testing for Trimethylamines (electronic sensor array)
- Testing for Sialidase Activity

“Decreasing Shelf-life” of *Trichomonas vaginalis* on Wet Mount Preparations

Survival of *T. vaginalis* over time



20% of wet mounts initially positive for *T. vaginalis* become negative within 10 minutes

Ref: Kingston MA, Bansal D, Carlin EM. Int J STD and AIDS 2003; 14: 28-29

Vaginitis Trichomoniasis

Incidence and prevalence

- **Most common treatable STD**
- **Estimated 3 million cases annually in the U.S.**
- **Approximately 3% prevalence in the general female population.**
- **Prevalence increases with age**
- **50%-60% prevalence in female prison inmates and commercial sex workers.**
- **Almost always sexually transmitted. Fomite transmission is rare.**
- **18%-50% prevalence in females with vaginal complaints.**
- **85% of women are asymptomatic.**
- **Not routinely tested in men.**

Vaginal Wet Preparation - Organisms



Trichomonas vaginalis

The arrowed objects in this photograph are *Trichomonas vaginalis*.



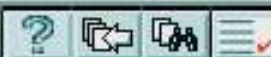
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Vaginal Wet Preparation - Organisms



Trichomonas vaginalis cont.

The arrowed object in this photograph is *Trichomonas vaginalis*.



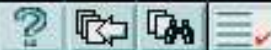
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Vaginitis Trichomoniasis

- ◆ Diagnosed by presence of motile Trichomonads in Wet Mount (urine in males). pH >5
- ◆ Culture is the Gold Standard (Diamond's TYI broth medium in glass tubes)
- ◆ *InPouch* system can be used for live and culture
- ◆ DNA Probes (problem with FP)
- ◆ Antibody-based technologies not really used

Yeast

Some yeast are normal in the vagina

Candida species are normal flora of skin and vagina

Yeast is more prevalent in pregnant women, diabetics, patients with catheters, and those on antibiotics

Vulvovaginal Candidiasis

(overgrowth of yeast)

Diagnosis

- ◆ History, clinical presentation and symptoms
- ◆ pH normal (4.0 - 4.5)
- ◆ Cultures not useful
- ◆ DNA probes
- ◆ Wet Mount

Vulvovaginal Candidiasis

Wet Mount:

- ◆ Presence of buds are oval shape and are paired, they resemble shoe prints, about the size of RBC's
- ◆ Presence of Pseudohyphae -- distinguished from true hyphae by their method of growth, relative frailty and lack of connection between the cells. Some true hyphae may also be present
- ◆ Increase in the number of WBCs is characteristic

Vaginal Wet Preparation - Organisms



Yeast/fungi

The arrowed objects in this photograph are yeast (hyphae and budding).



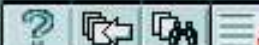
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Cytolytic Vaginosis

- ◆ A vaginal condition that involves an **overgrowth of Lactobacillus**, which is part of the normal vaginal environment
- ◆ Cytolytic vaginosis is not an infection or STD
- ◆ Symptoms include itching, redness variable discharge
- ◆ Etiology is unclear