



GEORGIA DEPARTMENT OF HUMAN RESOURCES

Division of Public Health



Microscopy for Public Health Nurses

DIVISION OF PUBLIC HEALTH

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Preface

This manual is designed as a training manual and resource for Georgia's district and county public health personnel on the fundamentals of clinical microscopy. It provides a basic overview of microscope function, use, and care, as well as applied microscopy for the diagnosis of sexually transmitted diseases (STDs). It reflects the current standards of practice within the Georgia STD Program. The use of manufacturer names or images is for illustration purposes only and should not be viewed as an endorsement of any particular product item.

Questions about Georgia STD Program activities should be directed to:

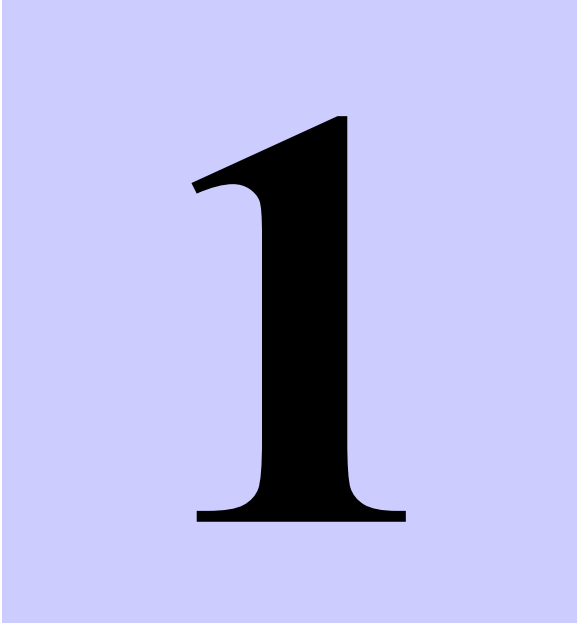
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1

Modern Microscopes

- *History*
- *Types*

History

The compound microscope occupies a revered place in the annals of microbiology as it has been at the front lines of discovery ever since it's invention around 1595. Such notables as Robert Hook and Jan Swammerdam built some of the earliest compound microscopes but it was not until 75 years later when the “Father of Microbiology,” Anton Van Leeuwenhoek, began making microscopes and studying the microscopic world.



Anton van Leeuwenhoek

A microscope is an instrument designed for viewing objects that are too small to be seen by the naked eye. The science of investigating small objects using such an instrument is called microscopy, and the term microscopic means minute or very small, requiring a microscope to be seen. “Microscope” is the combination of two words, “micro” meaning small and “scope” meaning view.



van Leeuwenhoek's microscope

Types

Several different kinds of microscope can be found in use in biomedical laboratories:

Compound Microscope: The most common type of microscope. It contains two or more lenses, hence the term “compound”, and utilizes visible light to produce a two dimensional image of an object viewed thru the oculars. Typical magnifications of a light microscope range from 50x to 1000x.



Compound light microscope

Stereo/Dissecting Microscope: A stereo microscope uses light from two different paths to produce a three dimensional view of the specimen. Stereo microscopes have high depth perception but low resolution and magnification. These microscopes are best used for dissecting and viewing large specimens i.e. whole insects.

Transmission Electron Microscope (TEM): The TEM utilizes magnets to focus a beam of electrons and pass it thru an object placed within the beam path to produce a two dimensional image. Samples for observation must be completely dry and no more than one cell thick, but may be viewed at magnifications of up to 200,000x.

Scanning Electron Microscope (SEM): An SEM also focuses an electron beam onto an object, but in this case, the focused beam knocks electrons from the objects surface which are then collected and reconstructed to provide an image of the objects surface. Samples must also be completely dry as with TEM, but may consist of an entire mosquito. Possible magnifications range from 15x to 200,000x.

Confocal Microscope (CM): This type of microscope utilizes one or more laser beams and “scanning mirrors” to rake the surface of the specimen with a point light of specific wavelength. Reflected or fluoresced light from the scan is then detected by the “scanning mirrors”, transmitted to a photomultiplier tube (**PMT**) through a pinhole (or in some cases, a slit), and the output from the PMT is built into an image and displayed by a computer. Laser scanning confocal microscopy has the ability to produce three dimensional images of specimens that have a thickness ranging up to 50 micrometers or more.



*Transmission
electron microscope*



Microscope Components

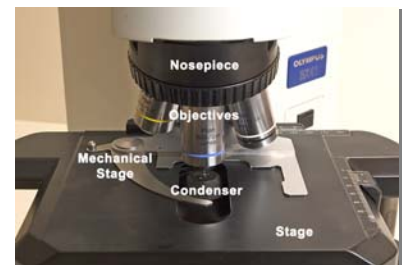
- *Mechanical*
- *Optical*
- *Exercise*

The compound microscope consists of mechanical and optical components which are both essential to the function and use of the microscope. Mechanical components provide a rigid and stable platform onto which are mounted the optical components in precise alignment to allow high magnification viewing of specimens.

Mechanical Components (Fig. 1)

If one looks at a typical compound microscope from the top down, the basic mechanical components are:

1. **Binocular Head** – sits on top of the stand and is equipped with two oculars (eyepieces); adjustable for setting individual inter-pupillary distance.
2. **Nosepiece** – revolving turret which carries the objective lenses.
3. **Arm** – solid support for the optical and mechanical parts of the microscope.
4. **Stage** – platform on which the specimen is placed; may be equipped with a specimen holder and mechanical stage for moving specimen around on stage.
5. **Condenser Carrier and Focusing Knob** – holds the condenser and allows it to be moved up and down for critical alignment of the light path.
6. **Focus Knobs (coarse & fine)** – mechanical means of focusing the microscope.
7. **Base** – supports the microscope
8. **Field Iris** – located in the base, it limits the area of the specimen that is illuminated.
9. **Illuminator** – contains light source and consists of mirror centering knob, bulb centering knob, and collector lens focus knob for critical alignment of light source; modern compound microscopes contain pre-set lamps which do not need centering and/or alignment.



Optical Components (Fig. 1)

Optically, a compound microscope consists of three components:

1. **Oculars (eyepieces)** – lenses which provide secondary magnification of the specimen image and project the image into the viewers eye; available in various strengths (10x/20x); available in “high-eyepoint” configuration for eye glass wearers; adjustable to viewers eyes.
2. **Objectives** – lenses which magnify (10x, 40x, 100x) the specimen and resolve critical elements of the specimen.



- a. Objectives are of two general types:
 - i. *Dry* – never allow oil or other fluid to get on the optical surface.
 - ii. *Oil* – must be used with immersion oil for maximum resolution; the oil actually becomes part of the optical path.



- b. Objectives have various magnification “power”
 - i. *10x (low power)* - Used primarily for slide scanning and to locate specimen elements or areas for more detailed examination. When scanning a slide, begin at one edge of the specimen and scan using a back-and-forth or up-and-down motion.
 - ii. *40x (high dry)* - Used to examine smaller elements that have been located with 10x objective. Most useful for identifying cells, yeast and parasites. Immersion oil is not used with this objective.
 - iii. *100x (oil)* – Used for critical viewing of specimen elements at maximum magnification. Focus is critical and may not be possible if

Lens Markings Explained

Plan	= planachromat
100x/1.25	= magnification/numerical aperture
Oil	= oil objective
∞/-	= infinity corrected/any coverglass thickness can be used

Modern objectives are “parfocal” meaning that when rotated into the working position, they will be in focus, and “parcentric” meaning that each new objective selected will be centered on the same specimen area.

the specimen is too thick. Immersion oil is always required with this objective to obtain clear specimen views, gather light from the specimen, and to optimize the optical path.

3. **Condenser** – focuses light onto the specimen; alignment is critical to the resolution of the microscope; condenser iris determines specimen contrast and depth of field.

To calculate image magnification, multiply the ocular magnification by the objective magnification:

$$10\times (\text{ocular}) \times 40\times (\text{objective}) = 400\times \text{magnification}$$

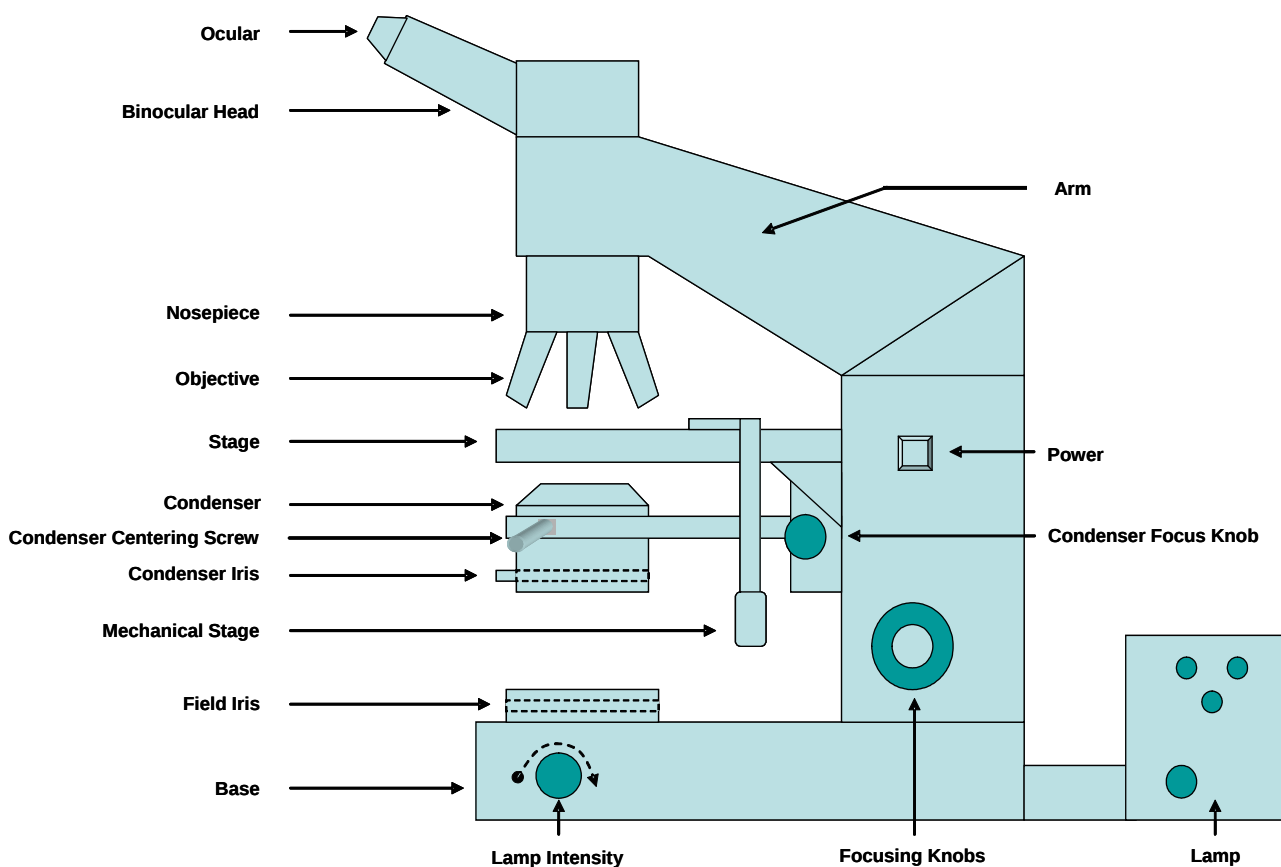


Figure 1. Compound microscope schematic identifying sixteen major components of the modern microscope.

Exercise 2

Identify the following components on your microscope:

Ocular

Binocular head

Arm

Nosepiece

Objective

Stage

Mechanical stage

Focusing knobs

Condenser

Condenser iris

Condenser focusing knob

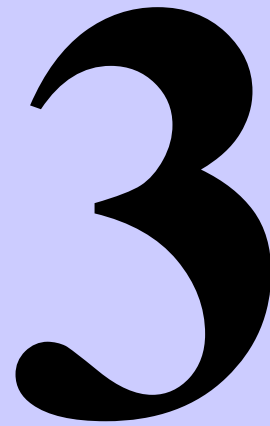
Condenser centering screw

Field iris

Base

Lamp

Power switch



Microscope Usage

- *General Procedures*
- *Ergonomics*
- *Microscope Set-up*
- *Using the Microscope*
- *Troubleshooting Problems*

General Procedures

Modern microscopes are precision instruments and should be treated as such. They must be kept clean and in alignment if they are to provide the expected results of the user. In general, you should:

1. Place the microscope on a table or bench which is stable and free from vibrations.
2. Plug the microscope into an outlet and secure any excess cord. Excess cord dangling over a table edge provides a possible “hook” with which to accidentally pull down the microscope.
3. When moving a microscope:
 - a. Lock the focusing knobs into position.
 - b. Always carry the microscope upright and close to your body. Never swing it or carry it one handed at your side.
 - c. Grasp the microscope firmly by the arm and place your other hand under the base for support.
 - d. Always carry the microscope with the 10x objective in the working position.
4. At the end of the day, clean all oil from the stage and oil objectives (see Chapter 5 – Cleaning), return the 10x objective or lowest power objective to the working position, and cover the microscope.

Ergonomics

The Occupational Safety and Health Administration (OSHA) has noted that "Microscope work is straining both to the visual system and the musculoskeletal system. Operators are forced into an unusual exacting position, with little possibility to move the head or the body. They are often forced to assume an awkward work posture such as the head bent over the eye tubes, the upper part of the body bent forward,

the hand reaching high up for a focusing control, or with the wrists bent in an unnatural position."

Microscope manufacturers are aware of these problems and continue to make advancements in these areas, but microscopists should pay particular attention to how they sit and work with their microscopes. Poor posture and body positioning are the chief causes of ergonomic injuries. Thankfully, most of these problems can be solved by simply changing positions or looking away from your intense gaze into the microscope.

To set up your microscope ergonomically, you should:

1. Sit in a comfortable ergonomic chair with adjustable height, back, seat, and arm rest controls.
2. Adjust your chair so that:
 - a. You feet are flat on the ground.
 - b. Your legs bend at a 90° angle at the knee.
 - c. The seat pan is raised so your eyes are at the level of the oculars.
 - d. Your elbows make a 90° angle to the table top and your hands can easily grasp focus, condenser, and mechanical stage knobs.
 - e. The seat back is comfortable and in a vertical position.
If your chair has a lumbar support, position it to support your lower back.
3. Position the microscope in front of you so that you do not have to lean-in to view thru the oculars.
4. Raise the light intensity until it is comfortable for viewing.
5. Finally, and most importantly, take frequent breaks and look away periodically. Looking away periodically (10-20 feet away) will exercise your eye muscles and getting up and



Sit comfortably at microscope with oculars at eye level, back straight, and arms at 90° angle to bench

moving will keep body parts from “falling asleep” and allow circulation to return to these areas.

Microscope Set-up for Optimal Viewing

The compound microscope should be “set-up” for optimal viewing every time it is used. Optimal viewing and resolution are only achieved after careful alignment of the microscope and setting up of “Kohler Illumination”. It is a technique, invented in the 1800’s by August Kohler, designed to fill the back of the objective lens with light and align all optical elements to provide an image of high contrast and resolution.

To set up Kohler illumination (Fig. 2), you will need to adjust the field iris, condenser, and condenser iris:

1. Turn the microscope on, place a specimen slide on the stage, adjust the light intensity, and focus, using the 10x objective.
2. Adjust oculars for your interpupillary distance and adjust ocular focus for your eyes; eyeglass wearers should leave their glasses on when using the microscope if it is equipped with “high-eyepoint” oculars.
 - a. Close your left eye (depends on the microscope) and focus the microscope.
 - b. Open your right eye and adjust the right ocular focusing collar to bring the image into sharp focus.
3. Close the field iris.
4. Raise (focus) the condenser using the condenser focusing knob until the image of the field iris becomes sharp in the ocular.
5. Center the field iris image using the condenser centering screws.
6. Open the field iris until the edges of the field iris go just beyond the field of view.



Compound microscope light path



Microscope parts involved in Kohler illumination

7. Remove one ocular and while looking down the tube at the opening of the objective, close the condenser iris all the way down.
8. Open the condenser iris until it is just inside of the field of view.
9. Replace ocular into the tube and adjust light intensity as needed.
10. Congratulations! You have just set up Kohler illumination.

***Kohler illumination should be re-checked
every time you change objectives to
maintain maximum resolution at all
viewing magnifications!***

Figure 2. Microscope Set-up (Kohler illumination)



1. Place specimen on stage; rotate 10x objective into place; focus specimen



2. Adjust inter-pupillary distance



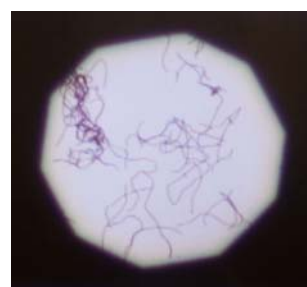
3. Close one eye; adjust ocular focus



4. Open eye; focus specimen



5. Close field iris



6. Focus field iris using condenser focusing knob



7. Center field iris using condenser centering screws; open to edge of field of view



8. Remove ocular



9. Close condenser iris



10. Look down ocular tube and open condenser iris to edge of objective opening



11. Replace ocular



12. Focus on specimen and begin scanning for organisms

Microscope Usage

Once you have set up Kohler illumination, follow these steps whenever using the microscope:

1. With the specimen slide securely in the stage specimen holder, begin to scan the slide under low power (10x) in a back-and-forth or up-and-down fashion going across the length of the specimen area.
2. When something of interest is seen, rotate the medium power (40x) objective into position
 - a. Center specimen of interest and refocus
 - b. Re-adjust Kohler illumination
 - c. Examine specimen
3. If higher magnification (100x) is required
 - a. Rotate 40x objective from viewing position
 - b. Place small drop of immersion oil onto specimen
 - c. Rotate 100x oil immersion lens into position and wait several seconds for air bubbles to clear
 - d. Center specimen and re-focus
 - e. Re-adjust Kohler illumination
 - f. Examine specimen
4. Return to low power and continue scanning specimen as needed
5. When specimen examination is complete
 - a. Rotate 10x objective into viewing position
 - b. Wipe oil from 100x oil immersion lens
 - c. Wipe oil from microscope stage (if any)
 - d. Turn off lamp
 - e. Cover microscope



Scanning slide with 10x objective



Examining specimen with 40x objective



Placing a drop of immersion oil onto specimen coverslip

Troubleshooting Microscope Problems

<u>PROBLEM</u>	<u>POSSIBLE CAUSES*</u>	<u>SOLUTION</u>
No light	1. Scope not plugged in 2. Scope bulb burned out	1. Check plug & reposition as needed 2. Check lamp and if you don't see light, replace bulb
Light flickers	1. Plug not secured in outlet 2. Bulb filament about to "blow" 3. Frayed cord 4. Faulty on/off switch	1. Reposition bulb 2. Replace bulb 3. Call service rep to replace frayed cord or faulty switch
Specimen appears dim	1. Microscope lamp is not bright enough 2. Specimen has stained faintly	1. Increase lamp intensity 2. Re-stain specimen
Specimen appears hazy, low contrast, or with fringes	1. Kohler illumination has not been properly set up	1. Set-up Kohler illumination
Specimen does not appear sharp	1. Ocular reticle has not been adjusted for your eyes 2. Oil may be on non-oil objective lens surface 3. Oil may have seeped under surface of oil immersion lens	1. Refocus ocular reticle 2. Clean objective lens surface 3. Call service rep for professional cleaning of inner lens optical elements
Specimen cannot be focused	1. Slide is upside down 2. Specimen is too thick 3. Coarse focus adjustment has been changed	1. Turn slide over 2. Make thinner specimen slide (smear, wet-mount etc.) 3. Select low power objective, position fine focus at half-way point, and focus using coarse adjustment; now return to high power and use fine focus
Cannot focus oil immersion lens	1. Specimen is too thick 2. Air bubble is on front of lens 3. Oil has seeped under lens optical elements	1. Make thinner specimen slide (smear, wet-mount etc.) 2. Rotate oil objective off of specimen, reapply small drop of oil, reposition oil objective 3. Call service rep for professional cleaning of inner lens optical elements
Cannot find specimen	1. Specimen spread thinly and/or widely 2. Specimen has stained faintly	1. Ensure that you have specimen area under viewing area 2. Look for a piece of debris to focus on 3. Move to edge of coverslip or slide and focus on the edge; then move back over specimen area

Microscope Care

- *General Procedures*
- *Cleaning*
- *Bulb Change*
- *Service*
- *Maintenance Logs*

General Procedures

Compound microscopes are wonderful yet delicate instruments which require regular cleaning and servicing to maintain their usability and highest levels of resolution. Unclean instruments will only hinder your ability to make accurate diagnoses and may even damage the microscope itself. It is therefore important to invest the time and money in both daily care and periodic professional servicing. Attention to daily care and prompt evaluation of problems can prolong the life of your microscope and prevent expensive repairs. In addition, it is always a good idea to give your microscope a thorough examination during routine cleaning.

To ensure optimal performance of your microscope, daily and annual maintenance should be performed and recorded in a service log.

Cleaning

For routine cleaning of the microscope mechanical and optical parts, use lint-free lens paper and lens cleaning fluid. Toilet paper, Kleenex, and paper towels should not be used as these may contain particulates that could scratch the lenses. **NEVER USE XYLENE OR ACETONE** to clean any part of the microscope as these will loosen the cement which holds optical elements together.

When not in use, microscopes should remain covered to prevent dirt and lint from settling on optical surfaces.

Objectives

1. Place the low power (10x) objective in the working position and remove your slide from the microscope stage.
2. Use lens paper lightly moistened with mild detergent or lens cleaning fluid to clean the stage, stand and base of the microscope as needed.
3. Use lens paper lightly moistened with lens cleaning fluid to gently wipe each objective.
4. Remove each lens in turn and:



- a. Inspect the lens surface to ensure it is free of oil and/or debris.
 - b. If oil or debris is seen, gently wipe the lens with lens paper lightly moistened with lens cleaning fluid in a circular motion, checking periodically to see that the material has been removed.
 - c. Visually inspect the surface of the lens, polish with lens paper as needed, and remount onto the turret.
5. To check objectives for nicks, cracks, and deterioration of the seal around the lens which would allow oil seepage into the lens:
 - a. Remove an ocular from the microscope and invert it for use as a magnifier.
 - b. Holding the ocular close to your eye and the objective lens surface close to the end of the ocular, examine the surface and undersurface of each lens for cracks or oil seepage.

Oculars (eyepieces)

1. Remove each ocular in turn from the binocular head and clean the surface using lens paper lightly moistened with lens cleaning solution. Wipe the lens surface in a circular motion and polish as needed.
2. Look into the bottom of the ocular tube and gently blow away any debris particles you observe.
3. Replace each ocular into the binocular head when finished.

Condenser

1. Lower the condenser, loosen the condenser locking pin, and slide out of its holder.



To clean oculars, wipe gently with lens paper in a circular motion

2. Inspect the surface of the condenser lens and condenser lens collar for oil and debris.
3. Clean the surface and collar using lens tissue lightly moistened with lens cleaning solution. Wipe the condenser lens surface in a circular motion and polish as needed.
4. Return condenser to condenser holder, lock into place, and raise it up into position.

Bulb Change

Microscope bulbs will blow out and will need to be changed.

However, most modern microscopes have pre-centered and aligned bulbs and you will only be required to remove the old bulb and put in the new bulb. Make sure you have the correct replacement bulb on-hand, before you need to replace it.

To change a bulb, consult your microscope manual. Your procedure may be different and/or simpler than the following:

1. Unplug the microscope.
2. Verify that you have the correct bulb. Do not use substitutes even if similar.
3. Remove any bulb alignment screws.
4. Remove bulb mount from the base of the scope.
5. Remove burned-out bulb (be sure the bulb is cool to the touch before handling).
6. Handle the new bulb with lens paper and insert into the bulb mount. Handling bulbs with your fingers will deposit finger oil onto the bulb and shorten the life of the bulb.
7. Remount bulb mount and replace screws if necessary. Center light if necessary.

***NEVER
TOUCH A HOT
BULB!***



Never touch the bulb with bare fingers as finger oil will shorten the life of the bulb

Microscope Service

The following companies can provide service for your microscope:

1. Atlanta Microscope: 770-998-2384
2. Southern Micro Instruments: 800-241-3312/770-956-0343
3. Southern Microscopes: 404-524-6334
4. C²orporation (Olympus Microscopes): 800-448-3929

Clean optics: oculars objectives condenser

Clean surfaces: stage arm

[illegible]

Clean optics: eyepieces, objectives, condenser

Clean surfaces: stage arm

Check bulb, wiring, mechanical parts, and alignment



5

Applied Microscopy

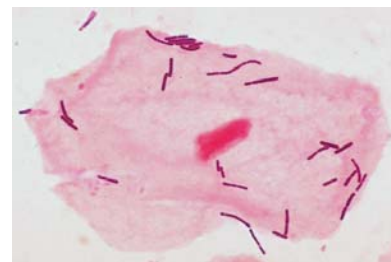
- *Diagnosis of Vaginal Infections*
- *Diagnosis of Male Urethral Infections*
- *Exercise*

Diagnosis of Vaginal Infections

To make an accurate diagnosis of a vaginal infection, it is important to understand the characteristics of the normal, healthy vagina and the delicate environment which exists inside it.

The Healthy Vagina

The vagina serves as a passageway between the outside of the body and the inner reproductive organs. In normal women of reproductive age, the vaginal acidic pH balance of <4.5 discourages infections from occurring. This acidic environment is created by a normally-occurring bacteria, *Lactobacillus*, a gram positive, non-sporeforming, rod-shaped bacteria.



*Epithelial cell with
lactobacillus (Gram stain)*

A healthy vagina produces secretions to cleanse and regulate itself similar to how saliva cleanses and regulates the environment of the mouth. These vaginal secretions are normal and all women have some discharge. Normal discharge may appear clear, cloudy white, and/or yellowish when dry on clothing. It may also contain white flecks and at times appear thin and stringy.

A variety of cell types may also be present with squamous epithelial cells (SEC) being the most prevalent. The SEC is a large polyhedral cell with a small nucleus and somewhat granular cytoplasm. “Clue” cells, seen in smaller numbers, appear larger as they are SECs which have become covered with numerous coccobacilli.



*Epithelial cells in
vaginal wet mount*

As many as five different species of bacteria may be present in vaginal secretions creating a delicate balance which when upset can create an environment conducive to infection.

Changes in normal discharge can occur for many reasons, including menstrual cycle, emotional stressors, nutritional status, pregnancy, usage of medications - including birth control pills, and sexual arousal. During the menstrual cycle pH of the vagina fluctuates and is least acidic on days just prior to and during menstruation. Increased wetness and clear discharge may occur around mid-cycle. Infections, tend to be most prevalent during this time of increased discharge and pH change.

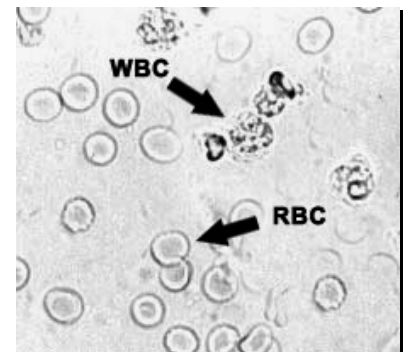
The Unhealthy Vagina

Changes in color or amount of discharge may signal an unhealthy vaginal environment and the beginnings of an infection. Vaginal infections are very common and most women will experience some form of an infection during their lifetime. Typical symptoms of vaginal infections are:

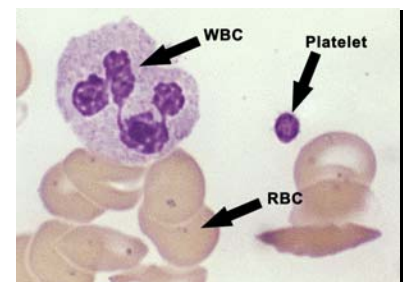
- Discharge accompanied by itching, rash or soreness
- Persistent, increased discharge
- Burning on skin during urination
- White, clumpy discharge (somewhat like cottage cheese)
- Grey/white or yellow/green discharge with a foul odor

When examining vaginal discharges for infection, the following are indicators for infection:

- Large numbers of clue cells - Bacterial Vaginosis (BV)
- Large numbers of white blood cells (WBC) - *Trichomonas vaginalis* (a protozoa **never** found in normal secretions), *Neisseria gonorrhea*, staphylococcus, Group B streptococcus, mycoplasma and less often with candida and BV.



RBCs and WBCs in vaginal wet mount



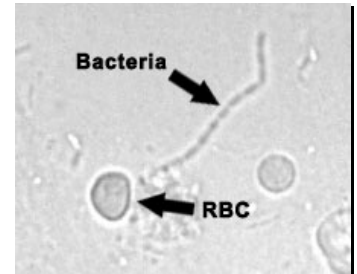
WBC, RBCs, and platelet (Wright's stain)

Common Vaginal Infections

Three of the most common vaginal infections are: bacterial vaginosis, trichomoniasis, and candidiasis.

1. Bacterial Vaginosis (BV)

BV is caused by a mixture of bacteria that multiply in the vagina when the natural acidic vaginal secretions are disturbed, but the reason for this is unclear. It is the most common abnormal vaginal condition. Similar to a yeast infection, there is an overgrowth of bacteria, and the delicate balance of the vaginal environment is upset when these bacteria occur in increased amounts. Recurrence of bacterial vaginosis is common and bacterial vaginosis can coexist with other vaginal infections.



RBCs and bacteria in vaginal wet mount

Patients with BV have a 5-fold increased rate of postpartum endometritis compared to those with normal vaginal flora. BV is also associated with a 3-fold increase in infections following hysterectomy, and postabortion PID. Additionally, patients with BV have a 40% increased rate of premature delivery.

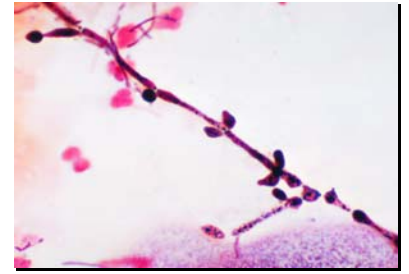
Signs and Symptoms: *Nearly half of the women with bacterial vaginosis do not display any symptoms.*

- Increased discharge
- Grey/white discharge
- Thin discharge
- Watery discharge
- Foul fishy odor

Treatment: There is no over-the-counter treatment. Numerous clinical studies have failed to demonstrate a benefit in treating sexual partners for BV and is not recommended by CDC. Therefore, BV is not really considered a sexually transmitted disease (STD).

2. Candidiasis

Candida infection is the second most common abnormal vaginal condition. It is estimated that 75% of women will have at least one episode of vaginal candidiasis during their child-bearing years. There is normally a small amount of yeast (*Candida albicans*) present in the vagina. A yeast infection occurs when there is an overabundance of yeast, often caused by a change in the pH balance of the vagina. Factors that may increase susceptibility to yeast infection include antibiotic use (protective bacteria are destroyed by antibiotics, allowing yeast overgrowth), diabetes, pregnancy, increased stress, use of oral contraceptives and immunodeficiency. Yeast infections are not usually sexually transmitted.



Candida albicans
(Gram stain)

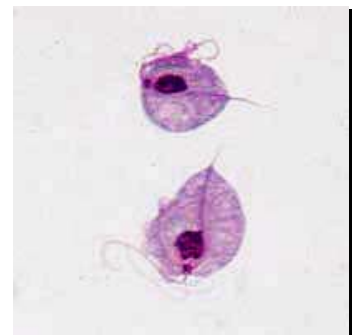
Signs and Symptoms:

- Perivaginal redness
- Perivaginal itching accompanied by burning and stinging sensations
- Increased discharge
- Cream-colored to pale yellow discharge
- Thick and curdy (cottage cheese-like) discharge

Treatment: Treatment is available and treatment of the patient's sexual partner does not decrease the frequency of recurrence. As with BV, vaginal candidiasis is not considered a true STD.

3. Trichomoniasis

Trichomoniasis is caused by a one-celled protozoan organism, *Trichomonas vaginalis*, which is almost always spread through sexual contact. It can survive for up to twenty-four hours in a moist environment, making wet towels or bathing suits possible agents of transmission.



Trichomonas vaginalis as
seen in vaginal wet mount

Signs and Symptoms: *Most men and some women do not display any symptoms.*

- Increased discharge
- White, gray, or greenish frothy discharge
- Foul odor
- Increased urination
- Inflammation of vulva/vagina
- Itching

Treatment: Trichomoniasis is considered a true STD, treatment is available, and treatment of sexual partners is necessary.

Laboratory Diagnosis of Vaginal Infections

In addition to the clinical presentation (physical appearance, odor, color, pH), laboratory diagnosis is critical to the identification of the source of vaginal/urethral irritation and discharge in female urogenital infections. Wet preparations and gram stains are the two most useful procedures at the time of clinic presentation with more sophisticated methods such as fluorescent antibody and nucleic acid amplification being used if wet preparation and gram stain results are inconclusive.

Wet Preparations

Wet preps are the most efficient and reliable diagnostic method to determine the source of vaginal irritation and discharge. It is a simple test in which samples of discharge are collected, tested for pH, and examined for the presence of bacteria, unusual numbers of cells (i.e. RBCs and WBCs), and microorganisms.

▪ Materials Required

- Lab coat, gloves, safety glasses
- pH paper or pH test strips
- Sterile cotton or Dacron swabs
- Test tubes (Saline Tube Method) – A red top vacutainer tube w/o gel may be used
- Physiological saline
- 10% KOH (potassium hydroxide)
 - Available, for example, from Remel: 1(800) 855-6730. (cat# 21230 for the 25 ml bottle; or cat# 21524 for individual dropper vials).
 - Store at room temperature and do not use beyond expiration date.
- Frosted end glass microscope slide for identifying patient slides
- Small (22x22 mm) glass coverslip
- Plastic, disposable Pasteur pipetter w/bulb
- Microscope with 10X and 40X objectives



Material used for making wet preps and checking vaginal discharge pH

▪ Procedure

1. Collect vaginal discharge samples using two swabs
2. Determine pH by applying a small amount of specimen onto a pH paper or pH test strip
3. Record pH on report form
4. Perform “Saline Tube” procedure
 - a. Place a swab into a test tube containing a small amount of sterile physiological saline.
 - b. Wearing gloves, mix the saline suspension and place a drop of specimen on a clean slide using a plastic disposable Pasteur pipette with bulb. Carefully place a #1 coverslip over the drop without trapping any bubbles.



Place a cover slip onto the specimen at a 45° angle to avoid trapping air bubbles

- c. Prepare another slide as above and add 1 drop of 10% KOH. The addition of 10% KOH helps in visualizing yeast due to lysis of cellular material. **NOTE:** addition of KOH may produce a “fishy” or amine odor. This is due to the metabolic breakdown of increased numbers of *Gardnerella vaginalis* and anaerobic bacteria.

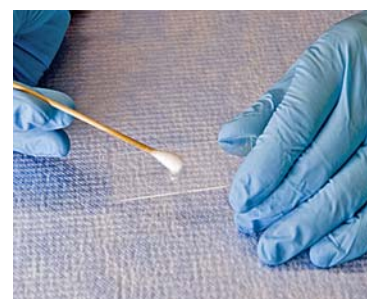
Record the result.

5. Perform “Direct Slide” procedure

- a. Prepare 2 slides, one with 2 drops of saline and one with 2 drops of 10% KOH.
- b. Using one specimen swab, rotate the swab 2-3 times in the saline drops and place a #1 coverslip over the suspension. The resulting suspension should be fairly light.
- c. With the other swab make 10-15 rotations in the 10% KOH drops for a fairly heavy suspension and place a #1 coverslip over the suspension. Record the presence or absence of a “fishy” or amine odor. The KOH will destroy most of the cellular material and allow better visualization of the yeast.

KOH slides should be examined last to allow time for cell lysis

Slide preps should be as thin as possible – preps that are too thick will not allow inspection of individual cells and will cause the cover slip to “float” around on the slide surface.



Rotate the specimen swab on slide two to three times and cover with cover slip

Microscopic Examination

- a. Position the specimen slide firmly in the slide holder.
- b. Select the low power (10x) objective and focus on the specimen.
- c. Scan the slide with the 10x objective by moving the slide in a back-and-forth or up-and-down motion to locate an area where individual cells can be seen.
- d. Switch to the 40x objective for closer examination and confirmation of trichomonas, clue cells, WBCs and normal SECs. Examine the KOH slide for yeast.



Scan slide up-and-back and from one side to the other

- e. If you have difficulty viewing cells due to low contrast, try closing the aperture diaphragm to lower the light, or lower the condenser a little until you see the cells outlined with a rim of light. Remember to re-set Kohler illumination if you alter the light path!
- f. Report results as numbers of organisms viewed per high power field (HPF) using the 40x objective. Ten fields should be viewed.



Examine specimen with 40x objective to identify clue cells, WBCs and SECs, yeast cells and hyphae, and microorganisms

Results and Interpretation

1. Bacterial Vaginosis (BV)

- a. Clue Cells
 - i. A few clue cells may be normal. More than 1 clue cell/HPF is considered abnormal.
 - ii. A clue cell is a SEC which has coccobacillus organisms clinging to its surface and at least 70% of the cell margin is obscured. NOTE: It is important to distinguish the clue cell from a SEC's normal granular appearance.
- b. pH – Greater than 4.5 due to the reduction of lactobacilli.
- c. Amine odor – “Fishy” odor due to production of amines from bacterial metabolism.
- d. Discharge – Homogenous, thin, gray or white.
- e. WBC's – Less than 1 WBC/SEC/HPF. The presence of many WBC's and clue cells may represent more than one infection.
- f. Diagnosis – Presence of any three of the four above (i.e. a to d).



Clue cells in vaginal wet mount

2. Candidiasis

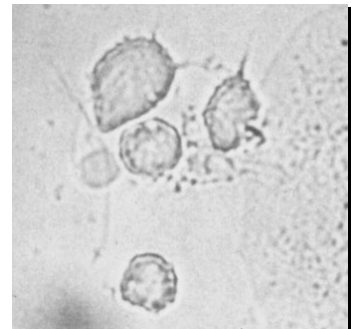
- a. Yeast organisms
 - i. Small numbers of budding yeast may be normal.
 - ii. The production of hyphae usually signifies tissue invasion and infection.
- b. pH – Less than 4.5
- c. Discharge – Typically referred to as “cottage cheese-like” but may vary from watery to thick.
- d. Amine odor – Negative.
- e. WBC’s Usually less than 1 WBC/SEC/HPF



Candida albicans in vaginal wet mount

3. Trichomoniasis

- a. *Trichomonas vaginalis*
 - i. Trichomonas is not part of the normal vaginal flora and when present always signifies infection.
 - ii. When examining the wet mount, the organism will be motile, producing a “jerking” motion. Sometimes the flagella can be seen. If examination is delayed over one hour, motility may be reduced or eliminated. The diagnosis of trichomonas cannot be made unless the typical motility is seen.
- b. pH – Greater than 4.5, sometimes as high as 6.0
- c. Amine odor – May be present
- d. Discharge – Excessive and may be greenish and frothy.
- e. WBC’s – Greater than 1 WBC/SEC/HPF



Trichomonas vaginalis
(Giemsa stain)

Differential Diagnosis of Vaginal Infections

	Normal	Bacterial Vaginosis	Candidiasis	Trichomoniasis
Microscopic	1. Many SECs 2. <1 Clue cells/HPF 3. Few budding yeast	>1 Clue cell / HPF	1. Hyphae 2. Budding yeast	Motile trichomonads
WBC	<1 WBC/SEC	<1 WBC/SEC	<1-1 WBC/SEC	>1 WBC/SEC
pH	< 4.5	> 4.5	< 4.5	> 4.5
Amine Odor	Negative	Positive	Negative	Neg or Pos
Discharge	None or white, clear	Thin, gray/white, homogenous	White, curdy	Yellow-green, frothy

Quality Control

- No commercial quality control is available.

Quality Assurance

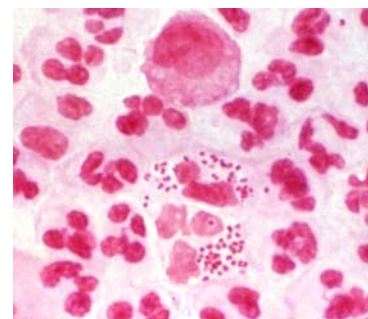
- In lieu of required QC for moderately complex tests, each laboratory that performs wet/KOH preps will participate in a Quality Assurance program.
- For each week of use, a wet/KOH prep will be examined by 2 different readers, and the results of each recorded and compared on a QA log.
- If the results are not comparable, the discrepancy is recorded and appropriate follow-up action is taken.

Laboratory Diagnosis of Male Urethral Infections

As with vaginal infections, it is important to remember that the normal male urethra does not exhibit any type of discharge except urination and ejaculation, and when one is observed, it is usually indicative of infection. Examination of male urethral discharge is generally performed to confirm or rule-out gonococcal infection.

Gonorrhea

Gonorrhea is a common sexually transmitted disease (STD) caused by *Neisseria gonorrhoeae* (GC). CDC estimates that more than 700,000 persons in the U.S. get new gonorrheal infections. It is a bacterium that grows and multiplies in the warm moist areas of the reproductive tract and urethra of both males and females. In addition, it can grow in the mouth, throat, eyes, and anus.



Neisseria gonorrhoeae
(Gram stain)

Transmission occurs through contact with the penis, vagina, mouth, or anus. Ejaculation does not have to occur for gonorrhea to be transmitted or acquired. Infections are transmitted more efficiently from an infected man to a woman (in 50 to 60% of instances of one sexual exposure) than from an infected woman to a man (in 35% of instances of one sexual exposure). Non-sexual human (skin to skin or skin to mucous membrane inoculation) or fomite transmission has not been documented. Gonorrhea can also be spread from mother to baby during delivery with the baby's eye being affected most often. People who have had gonorrhea and received treatment may get infected again if they have sexual contact with a person infected with gonorrhea.

There are three types of gonococcal infection:

1. Uncomplicated Infection – The majority of GC infections in this category are uncomplicated lower genital tract infections

caused by direct infection of mucosal membranes of the urethra, and endocervix.

2. Oropharyngeal and Anorectal Infections – Persons practicing receptive oral or anal intercourse may acquire oral or anal GC infections. Symptoms of oral GC may include pharyngitis. Symptoms of anorectal GC may include purulent discharge and burning or stinging pains during bowel movement. But, infections may also be asymptomatic.
3. Disseminated Infections – Approximately 1 to 3% of untreated gonococcal infections disseminate. In women the spread of infection throughout the reproductive organ result in a painful condition known as pelvic inflammation disease (PID). Dissemination may also manifest as arthritis, cutaneous lesions and septicemia.

▪ **Signs and Symptoms:**

- Men - Asymptomatic infections are estimated to occur in 10 to 50% of infected men. Some men have signs or symptoms that appear two to five days after infection. Symptoms can take as long as 30 days to appear and include:
 - Burning sensation when urinating
 - White, yellow, or green discharge
 - Painful or swollen testicles (sometimes)
- Women - generally do not have any symptoms.

▪ **Treatment:**

- Antibiotics can successfully cure gonorrhea in adolescents and adults.
- Drug resistant strains are increasing in many areas of the world. Persons with gonorrhea should be tested for other STDs

Gram Stains

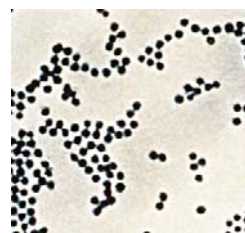
The Gram stain is the principle stain used for microscopic examination of bacteria. It is useful in the diagnosis of gonorrhea, candidal vulvovaginitis, and bacterial vaginosis, and in the assessment of urethritis, cervicitis, proctitis, and other infections characterized by mucosal discharge.

■ Principle

Hans Christian Gram devised the gram stain in the late 19th century and found that bacteria could be divided into two large groups based on the chemical nature of their cell walls. He found that those with cell walls containing a thick layer of peptidoglycans would stain deeply with crystal violet whereas those with thin peptidoglycans layers would not retain crystal violet during the staining procedure and be counterstained red with Safranin.

1. Gram Positive (+) - Those that take up the basic dye, crystal violet, and stain blue/purple.
2. Gram Negative (-) - Those that allow crystal violet to wash out with acetone/alcohol decolorizer and stain pink/red with safranin counterstain.

Bacterial Types



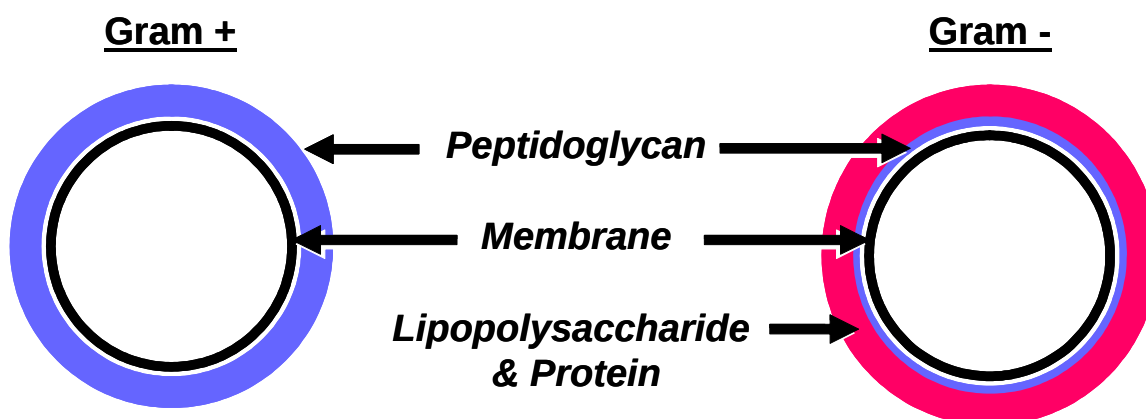
Cocci



Spirochete



Bacillus



■ **Materials Required**

- Microscope slides
- Alcohol or Bunsen burner
- Gram stain reagents:
 - Crystal Violet – primary stain
 - Gram's Iodine
 - Decolorizer (either 95% ethanol only; or 50:50 mixture of acetone and 95% ethanol; or Acetone only). The 50:50 mixture of acetone and 95% ethanol is the recommended choice.
 - Safranin – counter stain
- Sink or staining tray with water source
- Paper towels or blotting paper
- Immersion oil
- Bright-field microscope
- Material Sources:
 - Fisher Scientific: 800-766-7000; www.fishersci.com
 - Hardy Diagnostics: 800-266-2222; www.hardydiagnostics.com
 - Remel Inc: 800-255-6730; www.remelinc.com
 - Electron Microcopy Sciences: 215-412-8400; www.emsdiasum.com/microscopy



Gram stain components

■ **Procedure**

1. Prepare the smear
 - a. Carefully roll a specimen swab onto a small area of the slide to avoid disrupting cells.
 - b. Let the smear air-dry rather than drying it over a flame.
 - c. Heat-fix the smear briefly by passing it over a flame several times; the slide should feel warm but not hot.



Gently roll the specimen swab in the center of the slide

2. Stain the smear

- a. Flood the slide with crystal violet for approximately 30-60 seconds, then rinse with a gentle stream of tap water.
- b. Flood the slide with Gram's iodine for approximately 30-60 seconds, then rinse with a gentle stream of tap water.
- c. Rinse the slide with decolorizing solution until purple no longer runs from the thinnest part of the smear. The length of decolorizing depends on the composition of the decolorizing reagent and the thickness of the smear. For a 50% acetone to 50% ethanol solution, decolorize approximately 5 seconds.
- d. Flood the slide with safranin for approximately 30-60 seconds, then rinse with water.
- e. To dry the smear, blot it gently on a bibulous paper or clean paper towel (do not rub).



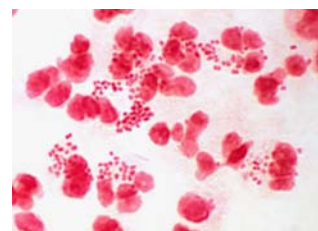
Gram staining should be performed over a sink to contain spillage

Microscopic Examination

1. Position the specimen slide firmly in the slide holder.
2. Select the low power (10x) objective and focus on the specimen.
3. Scan the slide with the 10x objective by moving the slide in a back-and-forth or up-and-down motion to locate an area where individual cells can be seen.
4. Switch to the 40x objective to identify material for more detailed examination.
5. Rotate the 40x objective from the working position and place one *small* drop of microscope immersion oil onto the specimen.
6. Carefully rotate the oil immersion objective (100x) into position and wait 1-2 seconds for oil to cover lens face and eliminate air bubbles.



Scan smear area with 10x objective to locate bacteria



Low power view of stained smear

7. Focus specimen using fine focus only.
 - a. If you cannot focus on specimen, you may be looking thru an air bubble – reapply another drop of oil and reposition lens to clear bubble.
 - b. If you do not see your specimen, slowly move the mechanical stage around to reposition specimen.
8. Use high power (100X), examine bacterial morphotypes and count PMNs/field.
 - a. Pink – bacteria (Gram -), cells, and mucus
 - b. Purple – bacteria (Gram +), yeast
9. Report results as numbers of organisms viewed per oil immersion field (OIF) using the 100x oil immersion objective. Ten fields should be viewed.



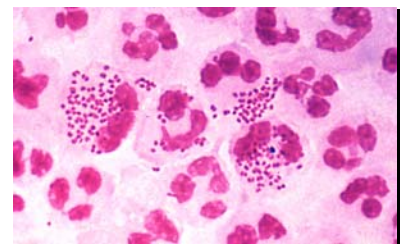
Place a small drop of oil on the smear for viewing with the 100x oil immersion objective

Results and Interpretation

Gonorrhea – microscopic examination of urethral discharges, if positive, reveal gram negative diplococci in WBCs. Properly prepared and interpreted gram stains of urethral exudates from males correlate with culturing at 98% sensitivity and close to 100% specificity. For men with asymptomatic infection, urethral smears have a sensitivity of 50% to 70%; for women, sensitivity is only about 50% and gram stain is not recommended.

1. Male

- Gonococcal Urethritis (GU)
 - ≥ 1 PMN with intracellular Gram-negative diplococci of typical morphology. Distinguish carefully between Gram-negative diplococci and Gram-negative rods.
 - Extracellular Gram-negative diplococci may also be present.
 - Numerous PMNs are usually present.



Neisseria gonorrhoeae (Gram stain)

- Non-gonococcal Urethritis (NGU)
 - Non-gonococcal urethritis (NGU) and non-gonococcal mucopurulent cervicitis (MPC) are caused by other sexually transmitted organisms and can complicate the clinical diagnosis of gonorrhea. The incidence of NGU exceeds that of gonorrhea and often coexists with gonococcal infections.
 - No intracellular GRAM NEGATIVE DIPLOCOCCI seen. NOTE: In early cases of infection, extracellular diplococci may be seen but are not diagnostic of GC.
 - Mononuclear cells and PMNs may or may not be present.

2. Female

- Gonococcal Urethritis (GU) – remove excessive mucous with a swab before collecting the specimen to reduce the amount of vaginal bacteria and cells in the smear.
 - ≥1 PMN clearly containing Gram-negative diplococci of typical morphology.
 - Numerous PMNs.
 - Extracellular Gram-negative diplococci.
 - Gram-negative rods and Gram-positive rods may be seen.
- Non-gonococcal Urethritis (NGU)
 - No intracellular Gram-negative diplococci.
 - Only extracellular Gram-negative diplococci found.
 - PMNs may be present.

Reporting of PMNs

Results are reported as numbers of PMNs viewed “per oil immersion field” or OIF using the 100x oil immersion objective. Ten fields should be viewed:

Rare	<1 HPF or OIF
Few	1-5 HPF or OIF
Moderate	6-10 HPF or OIF
Many	>10 HPF or OIF

Note: Five or more WBC/OIF is indicative of urithritis.

Quality Control (QC)

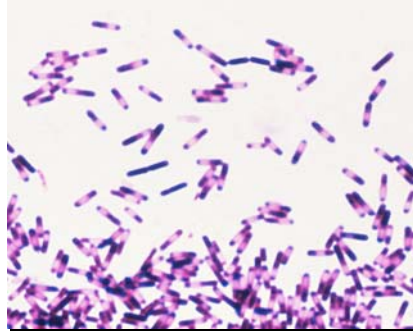
It is extremely important to perform quality control on all staining procedures to ensure accuracy and consistency. As a test of “moderate complexity” under CLIA, quality control tests should be:

- Performed each week of testing or when new bottles of reagent are opened.
- Performed using both positive and negative control slides. And,
- Recorded on QC sheet as illustrated on following page.

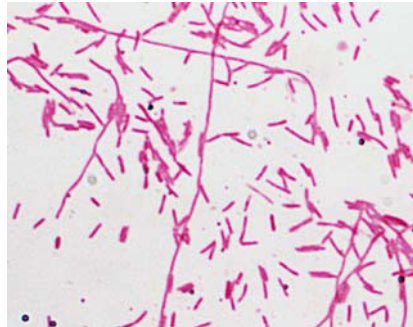
Positive and negative control slides must be examined before patient smears to verify the accuracy of the Gram stain

The positive control is *Staphylococcus aureus* and negative control is *Escherichia coli*. QC slides may be obtained from Remel Inc (Cat # 40140 & 40142) or Hardy Diagnostics (Cat # Z302).

1. **Positive Control (Gram positive)** - organisms stain blue/purple. Gram positive *C. difficile* is shown below.



2. **Negative Control (Gram negative)** - organisms stain pink/red. Gram negative *Legionella pneumophila* is shown below.



Gram Stain Weekly Quality Control Log

Reagent	Test Date	Lot #	Date Received	Date Opened	Expiration Date	Pos Control Results	Neg Control Results	Tech Initials
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								
Crystal Violet Iodine Decolorizer Safranin								

Expected test results:

Pos Control = Blue/Purple – report as Satisfactory (S); otherwise, report as Not Satisfactory (NS)

Neg Control = Pink/Red – report as Satisfactory (S); otherwise, report as Not Satisfactory (NS)

Control Slide Expiration Date: _____

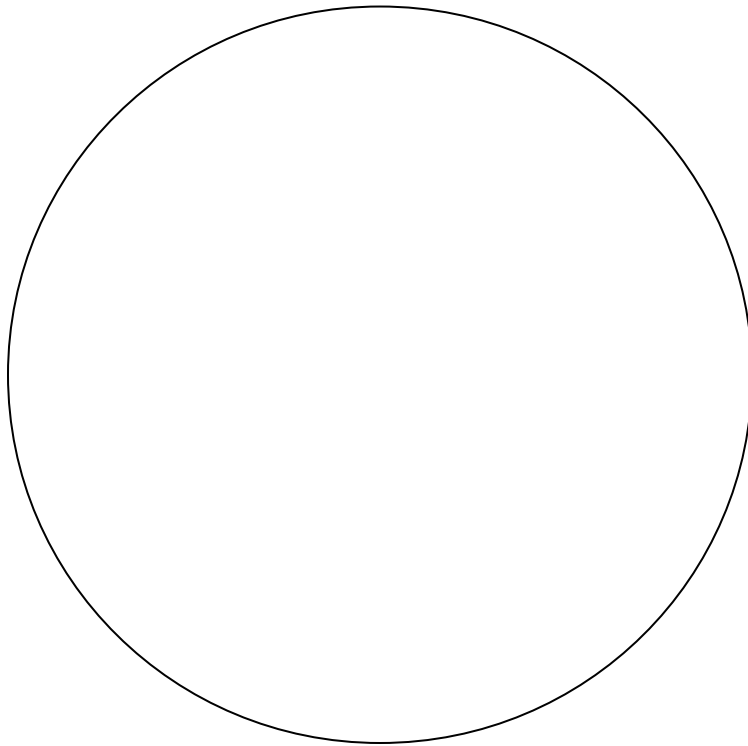
Troubleshooting Problems

<u>PROBLEM</u>	<u>POSSIBLE CAUSES*</u>	<u>SOLUTION</u>
Poor cellular morphology	1. "Scrubbing" rather than rolling the swab onto the slide 2. Overheating slide during staining	1. Gently roll the swab on the slide when preparing the smear 2. Monitor slide temperature during staining
No smear	1. Smear not properly heat-fixed to slide	1. Allow sufficient time for smear to "heat-fix" to slide
Poorly stained bacteria	1. Using old Gram stain solution 2. Over de-colorizing the slide	1. Use fresh Gram stain solutions 2. De-colorize for no more than 5 sec.
Incorrectly stained bacteria	1. Over de-colorizing the slide can make gram + bacteria appear gram -, and under de-colorizing the slide can make gram - bacteria appear gram + 2. Gram stain is contaminated with other bacteria and/or yeast	1. Be consistent in staining times 2. Do not under or over de-colorize 3. Use fresh Gram stain solutions
Bacteria are overstained and there are crystals in the smear	1. Smear was overstained 2. Smear was allowed to sit too long before being flushed 3. Smear was not de-colored long enough 4. Stains were made incorrectly	1. Staining times are critical to obtain correct results and should be followed exactly 2. Stain solutions should be made up correctly
Specimen slide is unidentifiable	1. Grease pencil or other soluble marker was used to identify patient slide 2. Slide was never labeled to expedite process 3. Writing on slide was not legible	1. All slides should be labeled <i>BEFORE</i> smears are applied 2. Use pencil or other non-soluble marker 3. Write identifying information neatly and legibly

*If you still cannot solve the problem, you may want to call your microscope service representative.

Exercise: Turn microscope on, select a slide, place it on the microscope stage, and:

1. Focus on specimen
2. Set up Kohler illumination
3. Select oil immersion objective
4. Carefully focus on specimen using fine adjustment
5. Draw a brief sketch of what you see



6. Report your observation
7. Calculate the image magnification

Report: _____ Rare Few Moderate Many

Image Magnification: _____ X _____ = _____ X

Appendixes

- *Laboratory Safety*
- *CLIA*
- *Definitions*
- *References*

Laboratory Safety

By their very nature, clinical laboratories are potentially hazardous work environments and laboratorians must be aware of the hazardous nature of the materials with which they work. Laboratories must have a laboratory safety manual and workers must be knowledgeable in effective work practices to keep themselves and others safe. In the case of microorganisms, laboratorians must be aware of infection control as it applies to obtaining patient specimens and then working with those specimens in the laboratory. As for chemicals, appropriate material safety data sheets must be available and all workers in the laboratory should be knowledgeable in emergency procedures for the chemicals in question.

Clinical specimens for STD diagnosis should be worked with using “standard laboratory practices.”

Standard laboratory practice forms the foundation for safety within the laboratory work environment. Every Federal and State regulation or guideline, is based on recognized “standard laboratory practice.” The CDC/NIH publication, “Biosafety in Biomedical and Microbiological Laboratories” (*BMBL*) and the National Research Council’s, “*Prudent Practices in the Laboratory*”, are recognized worldwide as providing lists of standard practices. So, what are standard laboratory practices? Quite simply, they are what should be performed by every laboratorian upon entering the laboratory to work, during their time working in the laboratory, and also upon exiting or removing material from one location to another. They are general working conditions for the laboratory, and, laboratory personnel and equipment designed to reduce or eliminate the possibility of persons (workers, co-workers, friends, family etc.) becoming exposed to hazardous materials. In addition, they provide supervisors with a list of responsibilities to apply to their workers.

ALL clinical specimens of human blood and body fluids should be regarded as potentially infectious per the Bloodborne Pathogens Standard.

At their “basic” level, there are about a dozen “standard practices” depending on how inclusive you make your definitions:

1. All laboratories should have a complete (standard practices, emergency procedures, exposure control plans, laboratory forms, MSDSs etc.) and up-to-date safety manual.
2. All lab workers should know the hazards present in the laboratory and do everything possible to limit their exposure to these hazards.
3. Safety devices (i.e. biological safety cabinets, fume hoods, centrifuges) should be serviced regularly and used per manufacturer's instructions. All users should be trained in proper working procedures before using the device.
4. Lab entry doors should be posted with warning signs and access restricted to those who work in the lab.
5. All laboratorians should don appropriate personal protective equipment (PPE) for the hazard being manipulated and never remove PPE from the laboratory.
6. Hazardous materials should be "contained" at all times especially during operations where there is a likelihood of creating an aerosol or splash.
7. Food of any kind should never be brought into the laboratory.
8. Fluids should be pipetted with mechanical devices and never by mouth.
9. Hands should be washed frequently, especially following glove removal and before exiting the laboratory.
10. The workspace should be kept neat and tidy, and decontaminated after all laboratory procedures.
11. All potentially contaminated materials should be decontaminated before removal from the laboratory



12. “Hazardous” materials should be placed into sealed secondary containers for transport out of the laboratory.

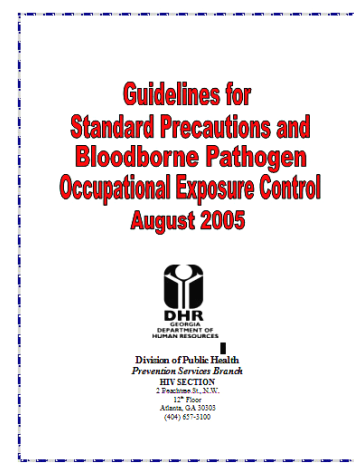
It should naturally follow after a review of these twelve, that each could be further expanded to a level of detail which should represent the laboratory safety manual. In addition, as risk elevates due to procedure (i.e. mixing, vortexing, centrifuging) or infective dose (i.e. culturing, concentrating), these standard practices should be elevated to provide greater worker protection and specimen containment.

OSHA Safety Requirements

The Occupational Safety and Health Administration (OSHA) and individual State standards require employers to provide a safe and healthy work environment for employees. Each work site must comply with OSHA standards pertinent to workplace hazards (23). Regulatory requirements for all OSHA standards, including specific information for medical and dental offices (24), are available at: [http:// www.osha.gov](http://www.osha.gov) and by telephone, 800-321-6742.

The OSHA Bloodborne Pathogens Standard applies to sites where workers have potential occupational exposure to blood and infectious materials (25). The requirements for compliance with this standard include, but are not limited to:

1. A written plan for exposure control, including post-exposure evaluation and follow-up for the employee in the event of an “exposure incident.”
2. Use of Universal Precautions, an approach to infection control in which all human blood and certain human body fluids are treated as if known to be infectious for HIV, hepatitis B virus, hepatitis C virus, and other bloodborne pathogens. Universal Precautions is one component of Standard Precautions, a



broader approach designed to reduce the risk for transmission of microorganisms from both recognized and unrecognized sources of infection in hospitals.

3. Use of safer, engineered needles and sharps.
4. Use of personal protective equipment (PPE) such as gloves and protective eyewear.
5. Provision of hepatitis B vaccination at no cost for those with possible occupational exposure who want to be vaccinated.
6. Safety training for handling blood, exposure to bloodborne pathogens, and other infectious materials.
7. Equipment for the safe handling and disposal of biohazardous waste (e.g., properly labeled or color-coded sharps containers and biohazard trash bags and bins).

Additional safety practices for performing testing are:

1. Prohibit eating, drinking, or applying makeup in areas where specimens are collected and where testing is being performed (i.e., where hand-to-mouth transmission of pathogens can occur).
2. Prohibit storage of food in refrigerators where testing supplies or specimens are stored.
3. Provide hand-washing facilities or antiseptic handwashing solutions.
4. Post safety information for employees and patients. Specific information on the Bloodborne Pathogens Standard and needlestick prevention is available at:

<http://www.osha.gov/SLTC/bloodbornepathogens/index.html>.



PPE should be selected based on the procedures being performed and the agents being worked with.

CLIA

Congress passed the Clinical Laboratory Improvement Amendments (CLIA) in 1988 establishing quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed. The final CLIA regulations were published in the Federal Register on February 28, 1992. The requirements are based on the complexity of the test and not the type of laboratory where the testing is performed. On January 24, 2003, the Centers for Disease Control and Prevention (CDC) and the Centers for Medicare & Medicaid Services (CMS) published final CLIA Quality Systems laboratory regulations that became effective April, 24, 2003.

CLIA requires all facilities that perform even one test, including waived tests, on “materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings” to meet certain Federal requirements. If a facility performs tests for these purposes, it is considered a laboratory under CLIA and must apply and obtain a certificate from the CLIA program that corresponds to the complexity of tests performed.

Laboratory tests are classified as waived (simple lab tests that have an insignificant risk of an erroneous result, i.e. urine pregnancy tests), or non-waived (moderate complexity, i.e. gram stains performed on endocervical or urethral specimens only, or high complexity). To determine which tests are categorized as waived or non-waived, refer to the lists of tests online at

<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/>

[cfCLIA/search.cfm](http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCLIA/search.cfm) or contact the Georgia Office of Regulatory Services at 404-657-5447. A list of State Agency addresses, phone numbers and contact persons is available online under the heading

Gram stains performed on endocervical or urethral specimens only are considered “moderate” complexity tests, whereas gram stains performed on other sources, i.e. bacterial cultures, are considered “high” complexity tests.

“State Survey Agencies (CLIA Contact List)” at the CMS CLIA website. If you do not have online access or have questions concerning certification, you may contact the CMS CLIA Central Office at 410-786-3531 for the address and phone number of your local State Agency.

Public health and clinical laboratories in GA must obtain a certificate of registration under CLIA. The type of certificate required depends upon the type and complexity of testing performed by the laboratory. The type of CLIA certificates laboratories may apply for are:

1. Certificate of Waiver (COW): Issued to a laboratory that performs only waived tests.
2. Certificate for Provider Performed Microscopy (PPM) procedures: Issued to a laboratory in which a physician, midlevel practitioner or dentist performs specific microscopy procedures during the course of a patient’s visit. A limited list of microscopy procedures is included under this certificate type and these are categorized as moderate complexity.
3. Certificate of Registration: Issued to a laboratory to allow the laboratory to conduct nonwaived (moderate and/or high complexity) testing until the laboratory is surveyed (inspected) to determine its compliance with the CLIA regulations. Only laboratories applying for a certificate of compliance or a certificate of accreditation will receive a certificate of registration.
4. Certificate of Compliance (COC): Issued to a laboratory once the State Department of Health conducts a survey (inspection) and determines that the laboratory is compliant with all applicable CLIA requirements. This type of certificate is issued to a laboratory that performs nonwaived (moderate and/or high complexity) testing.

The CLIA application (Form CMS-116) is available online at the CMS CLIA website. Forward your completed application to the Georgia Office of Regulatory Services.

5. Certificate of Accreditation (COA): Issued to a laboratory on the basis of the laboratory's accreditation by an accreditation organization approved by CMS. This type of certificate is issued to a laboratory that performs nonwaived (moderate and/or high complexity) testing.

The following exceptions to CLIA certification apply regardless of a laboratory's location:

- Any laboratory that only performs testing for forensic purposes.
- Research laboratories that test human specimens but **DO NOT** report patient specific results for the diagnosis, prevention or treatment of any disease or impairment of, or the assessment of the health of, individual patients.
- Laboratories certified by the Substance Abuse and Mental Health Services Administration (SAMHSA), in which drug testing is performed that meets SAMHSA guidelines and regulations. However, a CLIA certificate is needed for all other testing conducted by a SAMHSA-certified laboratory.

There are six CMS-approved accreditation organizations:

- American Association of Bioanalysis (AAB)
- American Osteopathic Association (AOA)
- American Society of Histocompatibility and Immunogenetics (ASHI)
- COLA
- College of American Pathologists (CAP)
- Joint Commission on Accreditation of Healthcare Organizations (JCAHO)

Contact information for the above CMS-approved accreditation organizations is available on the CMS CLIA web site at

www.cms.hhs.gov/clia. If you apply for accreditation by one of the CMS-approved accreditation organizations, you must apply to CMS for a COA concurrently. In Georgia, you can also contact the Georgia Office of Regulatory Services at <http://ors.dhr.georgia.gov/portal/site/DHR-ORS/> or 404-657-5447.

Definitions

Achromatic Lens - see objective

Aperture - opening; the diameter of the stop in an optical system which controls the ray bundle of light entering.

Aperture Diaphragm - either a rotating disc or an iris diaphragm on the condenser used to direct the appropriate wide/slender illumination cone to the specimen and entering the objective. Should never be used to regulate brightness. Resolution, contrast, and depth of field depend on the correct setting of the aperture diaphragm.

Bacillary - referring to bacilli or rod-like forms.

Bacillus - any rod shaped bacterium.

Brightfield - a microscopy technique in which light passes directly through specimen and into the objective, making specimen image appear dark against a bright background.

Brownian Motion - the random, dancing, zig-zag movements of minute, microscopic particles suspended in liquid. This motion is due to collisions of the particles with the individual random-moving molecules of the solvent.

Chlamydia - a genus of non-motile, Gram negative, obligately intracellular bacteria. They are quite small, and not normally observable with the light microscope.

Coccus/Cocci - a bacterial cell with a spherical shape.

Course Adjustment Knob - used for rapid or rough positioning of the specimen at the focal point of the objective lens.

Compound Microscope - a microscope made up of a 2-lens systems: (1) objectives which magnify the specimen, and (2) oculars which magnify the image produced by the objective lens.

Condenser - the lens system beneath the microscope stage, positioned to concentrate light correctly on the specimen and direct the light rays into the objective.

Brightfield - concentrates light on specimen, so that specimen contents alter the light, and project their dark image against a light background into the objective.

Darkfield - has a central stop, allowing only peripheral rays of light through as a hollow cone reaching an apex in the focal plane of the specimen. The only light getting through to the objective is reflected by specimen contents, and produces light images against a dark background.

Concave - a rounded, depressed surface.

Contrast - a relative difference between the brightest and darkest parts of the specimen; crispness. It is controlled by the aperture diaphragm. Too little contrast results in lack of definition; too much contrast reduces resolution.

Convex - a rounded, elevated surface.

Cover Glass - an ultra thin glass made to cover the specimen on the slide. It is a part of the image forming system. The cover glass has optical properties which are taken into account in computing and designing objectives. Manufacturers specify the thickness of the cover glass for general use on their microscopes.

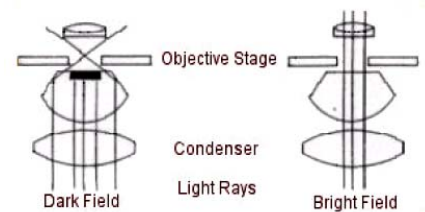
Dry Objectives - microscope objectives designed to be used dry, i.e. without oil.

Darkfield - a microscopy technique in which light is refracted by the specimen to produce a bright specimen image against a dark background.

Definition - the brilliance, clarity, distinctness, and sharpness with which the microscope magnifies and reproduces specimen detail.

Depth of Field - distance just above and below the focal plane--area being examined--that can be focused clearly. Depth of field/focus decreases as magnification increases.

Diaphragm - an adjustable device, usually thin, metal leaves, to expand or reduce the size of the light entry port of an optical lens system.



Döderlein's Bacteria - an obsolete and ill-defined term for aciduric, Gram positive rods commonly found in the vagina, which may be composed of mixtures of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus cellobiosus*, *Lactobacillus fermentum*, and even *Leuconostoc mesenteroides*.

Eyepiece - see ocular.

Field Diaphragm - the opening device (iris) that controls the aperture for the lamp condenser lens; alters the cross section of the ray bundle so that it coincides with the field of view for a large or small illumination area.

Field of View - the visible area through an in-focus lens.

Filters - used to control the intensity or colors of illumination.

Neutral Density Filters - a filter at the light source to control the intensity (brightness) of illumination.

Fine Adjustment Knob - exactly positions the specimen at the focal point of the objective lens.

Focal Length - the distance of the focus from the surface of a lens.

Focal Plane - the up/down level to which the specimen or object may be clearly imaged and studied.

Gram Stain - the standard staining procedure for the visualization and primary classification of bacteria. This procedure differentiates bacteria into two groups. The reaction of an organism is based upon the structural characteristics of the cell wall.

Gram negative - losing the primary stain or decolorized by alcohol in Gram's method of staining. Organisms appear pink at the completion of the staining process.

Gram positive - retaining the primary stain or resisting decolorization by alcohol in Gram's method of staining. Organisms appear purple at the completion of the staining process.

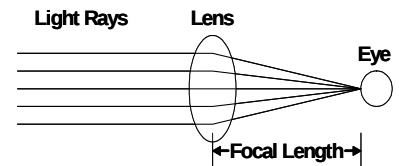


Image - a picture or conception.

Real - one formed by the collection rays in which the object is pictured as being inverted. Its presence can be viewed only by insertion of a receiving screen, etc.

Virtual - formed by a converging lens. The image seems to be situated on the same side of the lens as the object.

Immersion Oil - an oil with the same refractive index as glass, 1.515; used between the cover glass and an oil immersion objective to prevent scattering of light in air, and thus increasing resolution. Immersion oil becomes an optical component in the system.

Iris Diaphragm - adjustable assembly of thin metal leaves for varying the size of openings that determine the cross section of the light ray bundle entering the condenser and the objectives. Both the field and aperture diaphragms are iris diaphragms.

Köhler Illumination - a method of optimal illumination providing bright, evenly-dispersed, glare-free light with good contrast and resolution. The light beam is focused on the back focal plane of the condenser, the field diaphragm is focused in the field of view, and then the light is focused again at the back lens of the objective.

Lactobacillus - a single genus of the family Lactobacillaceae, occurring as large, Gram positive, anaerobic or microaerophilic bacilli. They are long, slender rods or pleomorphic in shape and motile. Normally encountered in the vagina.

Lens - a piece of glass or other transparent substance shaped to gather or scatter light rays, and used in the microscope and other instruments to magnify, increasing the visual acuity of the human eye.

Light - a radiant energy of the wavelength 400 to 700 millimicrons which upon reaching the retina of the eye stimulates nerve impulses to produce the sensation of vision. White light is composed of a mixture of colored light of various wavelengths.

Magnification - the number of times larger the image appears as seen through the microscope, than it appears to the eye at a distance of 10

inches (~ 25mm). The ratio, in diameters, usually is expressed as "power", "times", or "X".

Total magnification = magnification of eyepiece x
magnification of objective

Example: eyepiece = 10X objective = 40X

Total Magnification = (10X) x (40X) = 400X

Micrometer, Ocular - a glass disc inserted between the eyepiece and objectives that contains a measuring scale or defined grid; the scale will be visible superimposed on the field of view.

Stage - a precisely defined scale on a glass slide used for calculating the dimensional value of an ocular micrometer for each combination of ocular and objective.

Microscopy - the science of the uses and applications of microscopes. Two objectives of microscopy are forming a magnified image with as few optical defects as possible, and achieving good resolution and contrast. Contrast is based on the differential absorption of light between the specimen under study and its background; resolution is the ability to reveal and separate fine detail.

Mobiluncus - Gram-variable crescent shaped rods with a highly characteristic "tumbling" motility pattern.

Motility - having spontaneous but not conscious movement, contractility, ability of an organism to move in the medium, usually associated with the presence of flagella, cilia, or pseudopodia.

Neisseria - Gram negative cocci shaped bacteria, characteristically paired and shaped like coffee beans.

Nonspecific vaginitis - vaginitis which is often attributed to *Hemophilus vaginalis*.

Normal flora - the microorganisms that are more or less permanent residents of the superficial tissues. In any one body area, the microbial population is invariably mixed but the composition is remarkably stable. Although there are constant fluctuations in the minor

components, the same species remain numerically dominant in their own microenvironments. If the composition is altered by antibiotics or some other agent, the original composition is restored soon after the action of the drug or agent is removed. Consequently, the types of microorganisms most likely to be cultured from any healthy body site can be predicted, allowing for some variation due to factors such as age, diet and sanitation. Also called indigenous flora.

Numerical Aperture (N.A.) - a number, usually engraved on the objectives and condensers, expressing the size of the cone of light delivered by the condenser or collected by the objective. N.A. is defined by the formula:

$$\text{N.A.} = n \sin O$$

n = refractive index of medium between specimen and objective: in air, $n = 1.0$; in oil and glass, $n = 1.515$

$\sin O$ = the sine of $1/2$ the angle of the light cone entering the objective

The higher the N.A., the greater the resolving power, however the N.A. of the condenser must be = to the N.A. of the objective to achieve full resolving power of objective.

Objective - the lens system nearest the specimen used to magnify and direct image forming rays of the specimen to the oculars where they are further directed and magnified. Objectives are most important in determining the quality of the image produced.

Oculars - magnifying lens system of the microscope nearest to the eyes. Further enlarges the image produced by the objective.

Parfocal - the objectives are constructed so that the distance between the specimen and the image are the same for each objective, therefore you need to refocus only with the fine adjustment when changing from one objective to another.

Pleomorphic - occurring in various distinct forms; exhibiting pleomorphism; a common property in certain types of microorganisms.

Refractive Index - ratio of the speed of light in the first medium to the speed of light in the second medium. The use of immersion oil with oil immersion oil lenses prevents the loss of light via diffraction.

Resolution - the ability of a microscope to reveal fine detail in a specimen: crispness, clarity. The better the resolving power of a microscope, the closer two objects can be and still be distinguished as two objects.

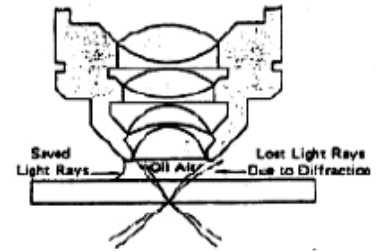
Staphylococcus - Gram positive cocci which exhibit a tendency to grow in bunches, pairs or tetrads.

Streptococcus - Gram positive cocci shaped bacteria with a tendency to grow in pairs or chains.

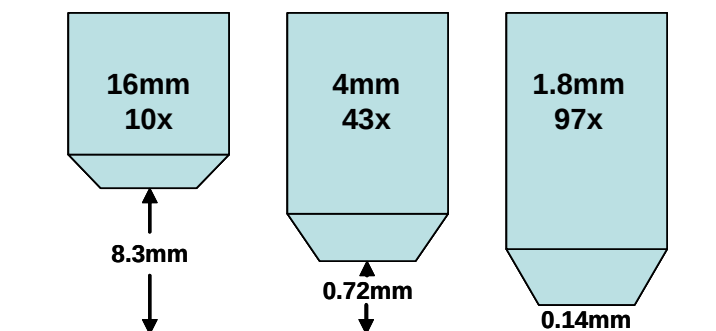
Vaginitis - an inflammation of the vagina (with or without purulent discharge) that is characterized by irritation of the vulva, pain with urination or with sexual intercourse, and itching or burning sensations. Vaginitis is an infectious process caused by the presence of one or more microorganisms and accompanied by the presences of white blood cells (leukocytes).

Vaginosis - a vaginal discharge characterized by remarkably abnormal quantities of both anaerobic and aerobic bacteria, however an increased number of white blood cells are not present (no inflammatory response).

Working Distance - the distance between the cover glass (or specimen if uncovered) and the tip (from lens) of the objective. Note that the working distance of objectives decreases with magnification increase.



Immersion oil on the left side Prevents light loss due to diffraction.



Note: Working distance of objectives decreases as magnification increases.

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- **Microscopy**
 - **Web Sites**
 - <http://www.amateurmicroscopy.net/>
 - <http://www.microscopyu.com/>
 - <http://www.biologie.uni-hamburg.de/b-online/e03/03.htm>
 - <http://www.microscopy.org/>
 - <http://www.olympusmicro.com/>
 - <http://www.zeiss.com/micro>
 - **On-line Training**
 - <http://www.med.unc.edu/microscopy/classes.htm>
 - <http://www.olympusmicro.com/>
 - <http://www.zeiss.com/micro>
 - <http://www.microscopyu.com/>
 - **Books**
 - Exploring With the Microscope: A Book of Discovery & Learning, Werner Nachtigall

- Microscopes: Bringing the Unseen World into Focus, Gail B. Stewart
- Fundamentals of Light Microscopy and Electronic Imaging, Douglas B. Murphy
- Basic Methods in Microscopy: Protocols And Concepts from Cells, a Laboratory Manual, David L. Spector, Robert D. Goldman
- The Microscope and How to Use It, George Stehli
- Introduction to Light Microscopy (Royal Microscopical Society Microscopy Handbooks), S. Bradbury
- Microscopy and Photomicrography: A Working Manual, Robert F. Smith
- Light and Electron Microscopy, Elizabeth M. Slayter, Henry S. Slayter