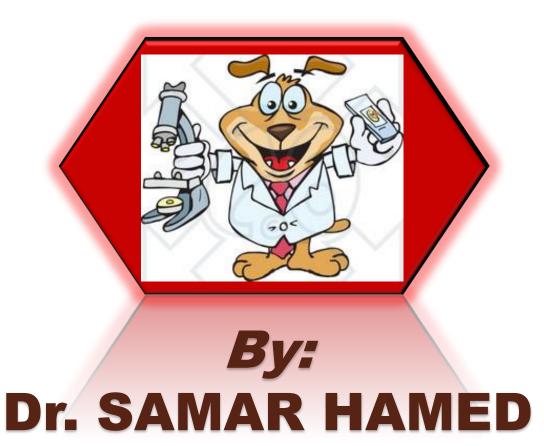
Lab (1) Pharmaceutical Microbiology Practical course.

For 1st year, 2nd semester

OBSERVING MICROORGANISMS THROUGH A MICROSCOPE



Identification of microorganisms

Microscopical examination.



> Culture.



Biochemical examination.





Microscopical examination



Microorganisms are <u>too small</u> to be seen by nacked eye, SO:

Microscope



Must be used to see those microorganisms





There are several types of microscopes but which used in lab is called:



Compound bright field light microscope.





Compound bright field light microscope

Compound: has 2 types of lenses (objective&ocular).

Bright field: examination of sample against bright field background.

Light: light as source of illumination.





Parts of light microscope:

Base: supports & stabilize the microscope.

Stage: on which slide rests.

has clips>>>hold glass slide

Has hole >>> allow passage of light to slide.

Body tube: connect ocular lens to revolving nose piece.

Arm: connect body tube to stage & to hold microscope.

Source of illumination: visible light may be;

built in source>>> lamp
 day light >>> mirror

<u>Iris diaphragm:</u> control amount of light entering conden specimen.

Condenser: focuses light to apoint just above the stage.

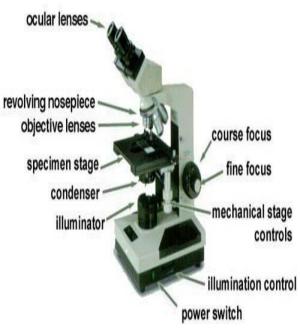
Focus adjustment knobs: move stage

course knobe>>> observable movement

fine knobe>>> non observable movement

Lens system:

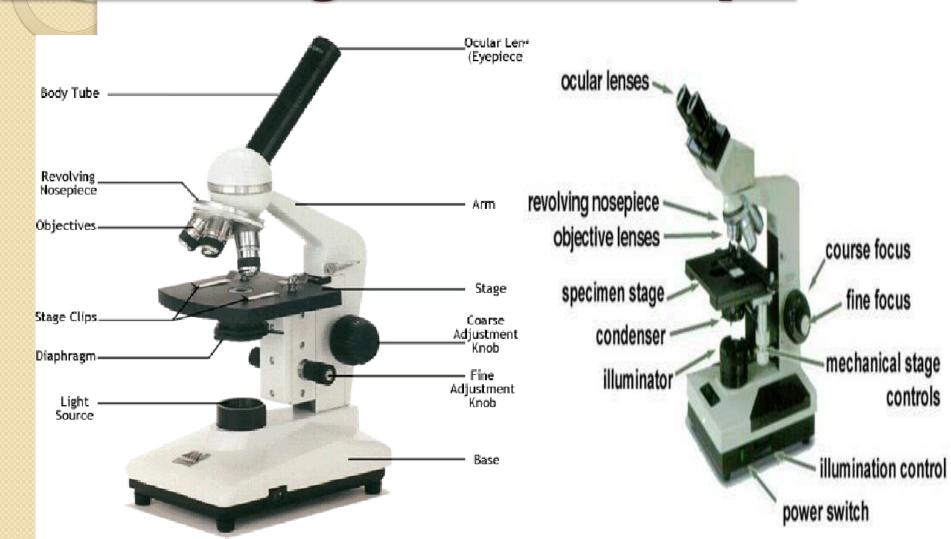








Parts of light microscope.







... Parts of light microscope:

Lens system: 2 types (objective & ocular):

Objective lenses:

- ✓ carried on revolving nose
- √ make initial magnification
- ✓ Low power lens(10x), high power lens(40x) & oil immersion lens(100x)

Ocular lens:

✓ Magnifies the image formed from objective lens (10x)

N.B: we will use oil immersion lens for examination of bacterial samples because:

- ✓ Refractive index of oil & glass = 1.5 >>> **so** prevent light distortion & give sharp image.
- ✓ Refractive index of air = I >>> **so** light beams will fall away from lens.





Magnification of microscope:

- ✓ by 2 lens system(objective & ocular)>>> dual magnification.
- \checkmark oil immersion lens give maximum magnification (1000x) \checkmark

Total magnification=
objective lens mag. X ocular lens mag.
(10 or 40 or 100x) X (always 10x)

Resolution power (R.P) of microscope:

- ✓ ability to distinguish fine ditails & recognize closely spaced objects as separat entities.
- ✓ R.P = 0.2 µm ????????





Stain (dye): chemical compound has chromophore (carry color).

<u>Cause of use:</u> bacteria is colorless & examind against bright background.

Importance of use:

- ✓ Make M.O visible.
- ✓ Study M.O morphology (size, shap, arrangement).
- ✓ Identify special types or special structures of bacteria.

Stainining may be:

- ✓ Simple staining: one stain type.
- ✓ Differential staining: 2 or more stain type & categorize cells into gps.

Types of stains:

- ✓ Basic stain
- ✓ Acidic stain
- ✓ Neutral stain





Types of stains (dyes):

Basic stain (cationic stain):

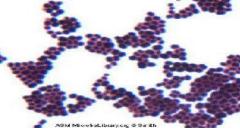
- Carry **+ve** charge.
- Bacteria carry **-ve** charge so attract the basic stain (**+ve** charge).
- Bacteria stained against colorless back ground(washing excess dye).
- Direct or positive stain.
- Ex: Crystal violet & Safranin.

2. Acidic stain (anaionic stain):

- Carry **-ve** charge.
- Bacteria carry **-ve** charge so repulse acidic stain (**-ve** charge).
- Bacteria unstained against stained background (no washing but air drying).
- Negative stain.
- Ex: Nigrosin.

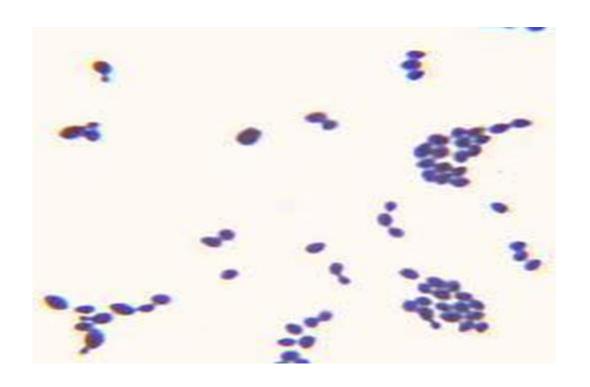
3. Neutral stain:

- For parasite staining.
- Ex: leishman stain.



1st lab

** Observing Yeast stained by crystal violet under microscope



- loop
- T.T holder
- T.T rack
- slides



- Permanent marker
- فوطة صفراء البالطو •









Lab (2) Pharmaceutical Microbiology Practical course.

For 1st year, 2nd semester

Simple Stain



Dr: SAMAR HAMED





Bacterial cells are colorless so need to be stained before microscopical examination.

> <u>Requirments :</u>

- √ Bacterial suspension (B.S).
- ✓ Stain.
- √ Flame.

Before saitning :

- √ Prepare the smear (heat fixed film).
- √ Fix specimen on slide by heat fixation process
 (Bunsen burner).
- Now, specimen is ready to be stained.
- Add dps from the desired stain on fixed smear.
- Wait until desired time passed.
- >Then examin under the microscope.







SIMPLE STAINING



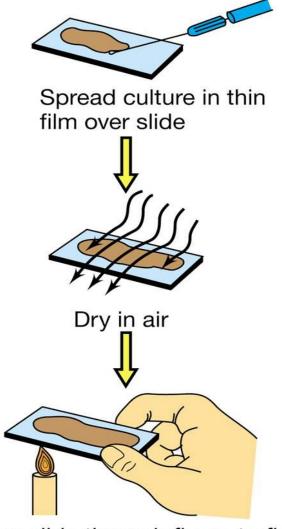


Procedure:

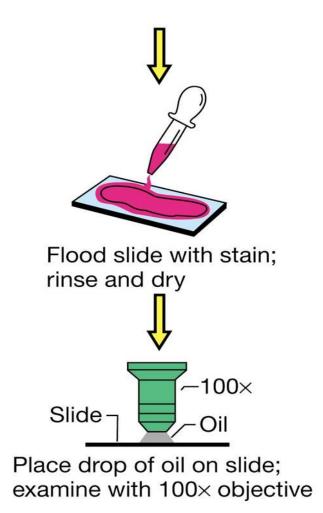
- 1) Put 4-5 loopful from B.S on slide (target circle).
- Prepare heat fixed film.
- Cover the fixed smear with 2 dps of crystal violet for 1 min
- 4) Rinse (wash) off the dye carefully under fine water flow.
- Leave slide until dry.
- 6) Add <u>1 dp</u>of oil on staind smear & spread it.
- 7) Examine under light microscope with oil immersion lens (100x)





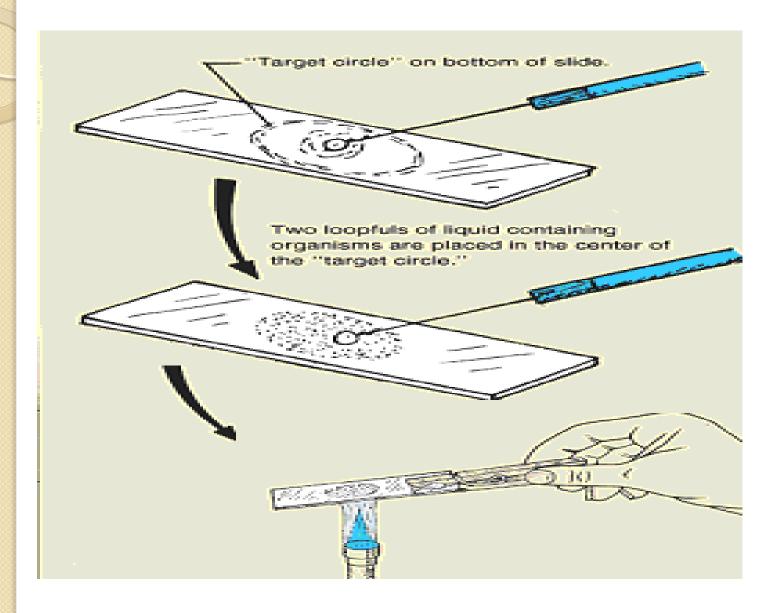


Pass slide through flame to fix



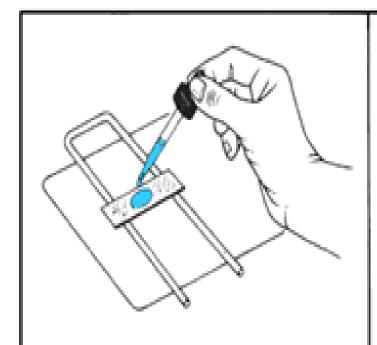




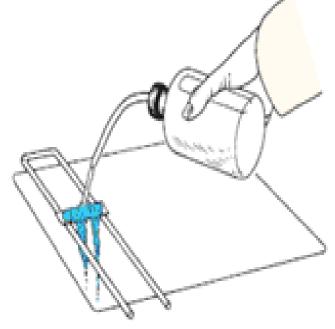








2 A bacterial smear is stained with methylene blue for one minute.



3 Stain is briefly washed off slide with water.







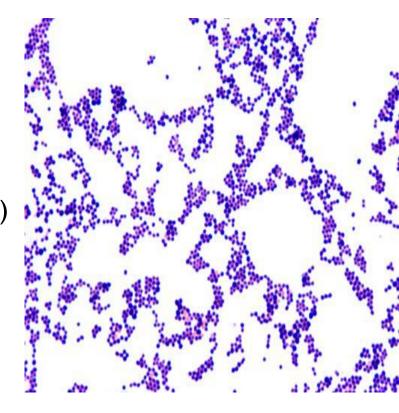




Name: Staphylococcus aureus.

Shape: Spherical

Arrangement: Bunch (grape like)







Name: Bacillus subtilis

Shape: Long rods

Arrangement: Chain



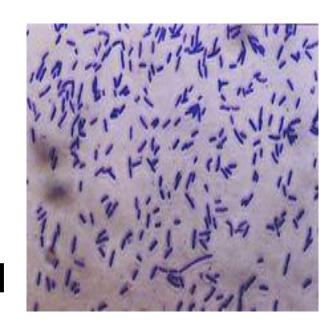




Name: Escherichia coli

Shape: Short rods

Arrangement: Single scattered







Name: Saccharomyces cerevisiae (Yeast)

Shape: Oval

Arrangement: Single scattered

