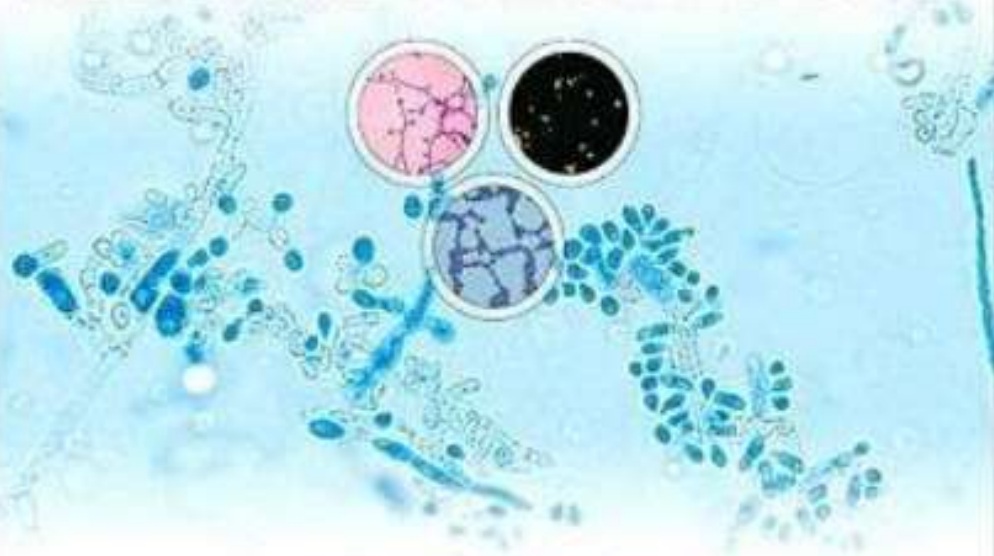


BAILEY & SCOTT'S

DIAGNOSTIC MICROBIOLOGY



TWELFTH EDITION



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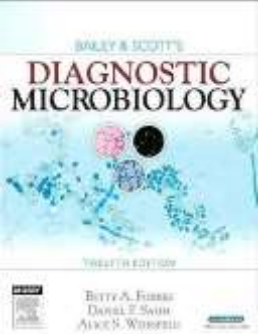
2/5/2014

Diagnostic Microbiology

320 MIC

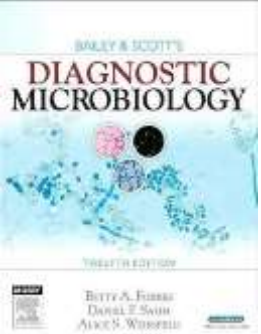
Lecture: 1

Identification of Microbes



Outlines

- What is expected out of this course???
- At the end of this course you will be able to apply....
 - Conventional / Microbial
 - Diagnostic Microbiology Techniques
 - Some basic principle microbiology testing
(Follow a specimen through the lab.)
- Task of the methods –
To make the microorganisms visible & measurable.



Major Groups of The Microbial World

- Bacteria
- Fungi
- Algae
- Protozoa
- Viruses

Major Features:

- ✓ Small
- ✓ Diverse (Appearance , Genetics)



The basic Principle of Microbial Diagnosis

- Clinical assessment.
- Collecting & transporting specimens.
- Microscopy.
- Culture.
- Some basic principles of microbiology testing.
 - Culture sensitivity.
 - Non-cultural diagnostic methods.
 - Virological diagnosis.



Methods of Microbial Diagnostic

The methods microbiologist use fall into two categories:

- **Phenotypic:** Morphology (micro- & macroscopic).
- **Genotypic:** Genetic techniques.



Phenotypic Protocols

- **Phenotypic Characters:**

- a) Microscopic /Macroscopic Morphology
- b) (wet preparation – staining properties – growth on different laboratory conditions media).
- c) Metabolic differences (biochemical reactions).
- d) Animal pathogenicity.
- e) Bacteriophage typing.
- f) Serology.
- g) Fatty acids analysis.

Disadvantages:

Poor discriminatory.

Difficulties in typing.

Not provide enough information about microorganisms for today's needs.



Phenotypic Protocols

- **Phenotypic Methods:** “Old Fashioned” methods via biochemical, serological, and morphological are still used to identify many microorganisms.
- Use purely biological phenomena.
- Examining specimens to detect isolate and identify pathogens.



Phenotypic Methods

A- Morphology:

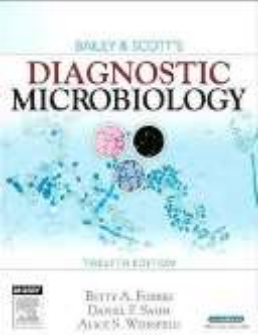
Microscopic: cell shape & arrangement- microscopic structures & characteristics.

Macroscopic: traits that can be assessed with naked eye.

e.g. appearance of colony including shape, pigments, speed of growth, and growth pattern in broth.

B- Physiologic / Biochemical characteristics are traditional for bacterial identification.

These include enzymes (catalase, oxidase, decarboxylase), fermentation of sugars, capacity to digest or metabolize complex and sensitivity to drugs can be used in identification.



Phenotypic Methods

C- Animal Pathogenicity: to detect toxins and virulence.

D- Phage typing: procedure for characterizing and detecting single bacterial strains by their reaction (susceptible or resistant), may used in outbreak infections.

E- Immunological: serological tests.

F- Fatty acid profiles: routinely used to identification of mostly anaerobic bacteria, non fermentative gram, negative, yeast.



A- Morphology:

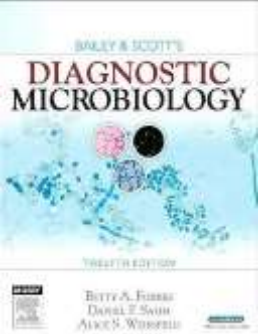
1- Microscopy: Microorganisms can be examined microscopically for:

a) Size

b) Bacterial motility: e.g. hanging method.

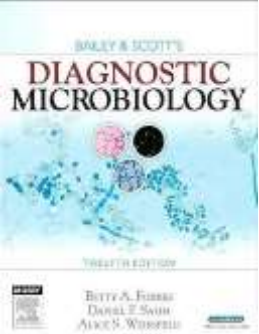
c) Shape, Morphology, and Staining reactions of Bacteria:

- I. **Simple stain: one dye is used e.g. Methylene blue stain (reveals shape, arrangement or cell grouping).**
- II. **Differential stain: 1- Gram stain: differentiation between G +ve and G -ve bacteria**
 - Primary stain (Chrysal violet).
 - Mordant (Grams iodine).
 - Decolorization (Ethyl alcohol).
 - Secondary stain (Safranin).



A- Morphology:

- Some microbes have unique characteristics that can be detected with special staining procedures:
 - III. Acid-fast reaction (Ziehl-Neelsen stain):** staining acid fast bacilli
 - Carbol fuchsin with heat.
 - Decolorization (HCL & methyl alcohol).
 - Counter stain (methylene blue).
 - iv. Spore stain:** endospore staining.
 - v. Structural stain:** reveal certain cell parts not revealed by conventional methods:
 - Special characters: capsules (capsule stain), flagella stains, and granules (volutin).



Specimen Preparation

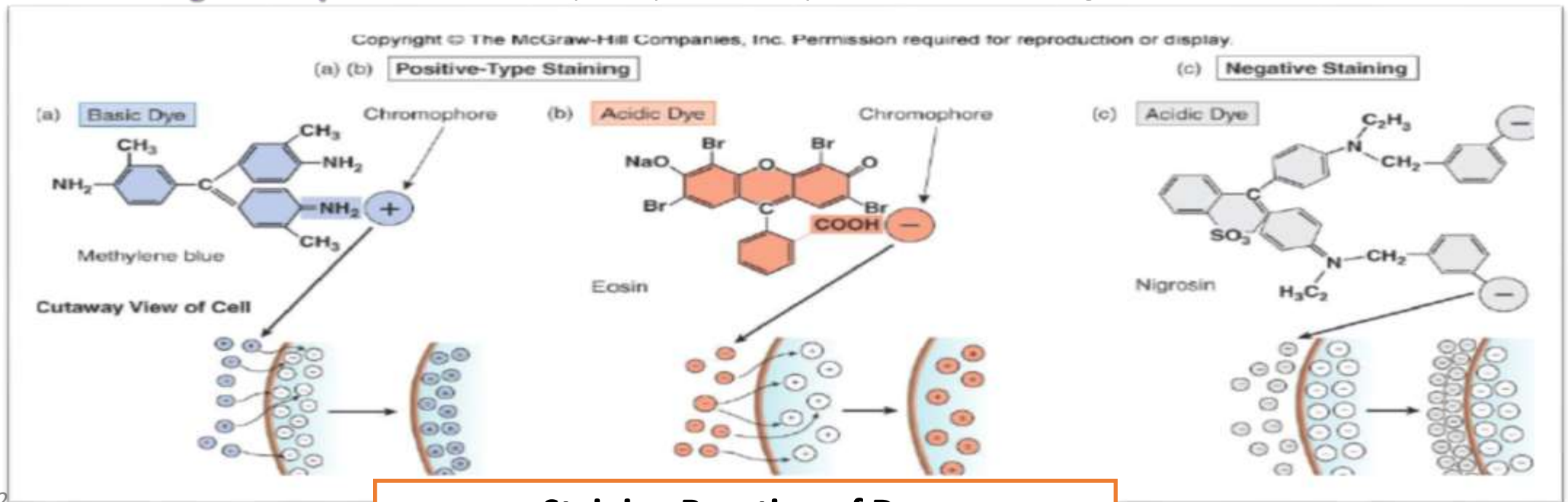
- **Wet Mounts and hanging drop mounts:** allow examination of characteristics of live cells (size, motility, shape, and arrangement).
- **Fixed Mounts:** are made by drying and heating a film of specimen.

This smear is stained using dyes to permit visualisation of cells or cell parts.

Staining

Dyes create contrast by imparting a color to cells or cells parts.

- **Positive dyes:** surfaces of microbes are negatively charged and attract basic dyes.
 - **Basic dyes:** cationic, positively charged chromophore.
 - **Acidic dyes:** anionic, negatively charged chromophore.
- **Negative dyes:** microbes repel dyes, the dye stains the background.

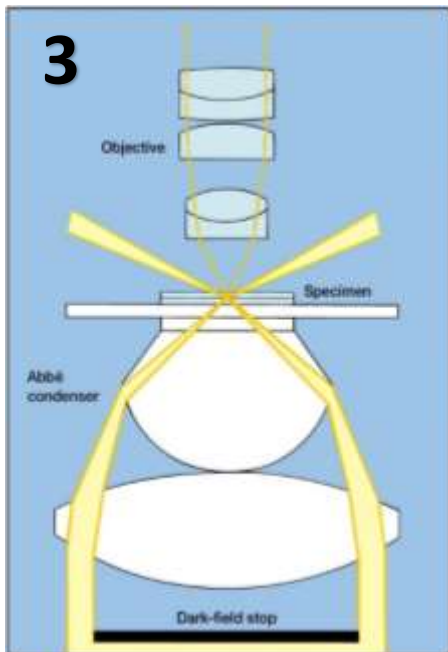


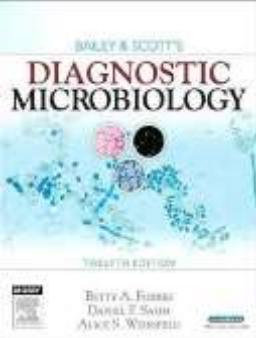
Staining Reaction of Dyes



Types of Microscope

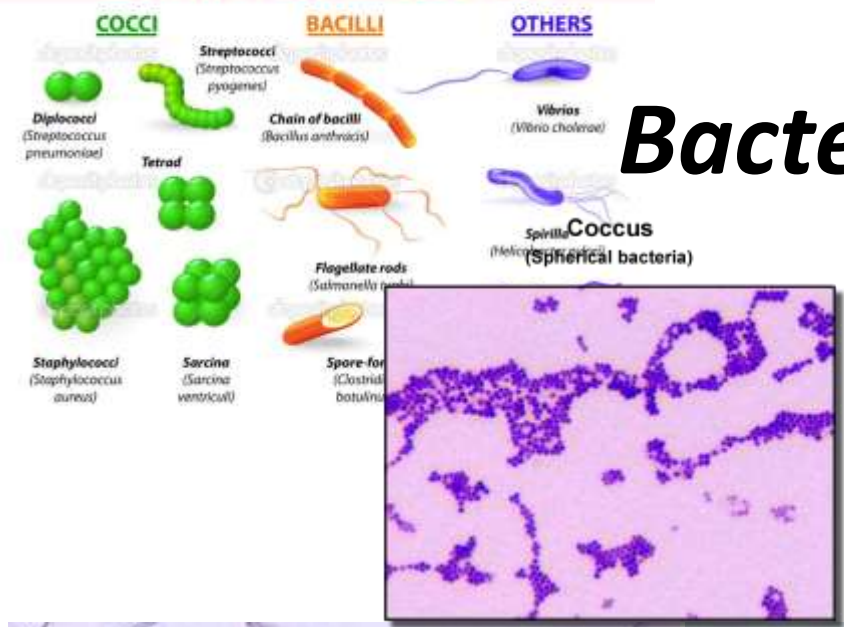
- 1- Light microscope (Digital).
- 2- Stereo microscope (Dissection).
- 3- Dark field Microscope.
- 4- Electron Microscope (Scanning & Transmission).



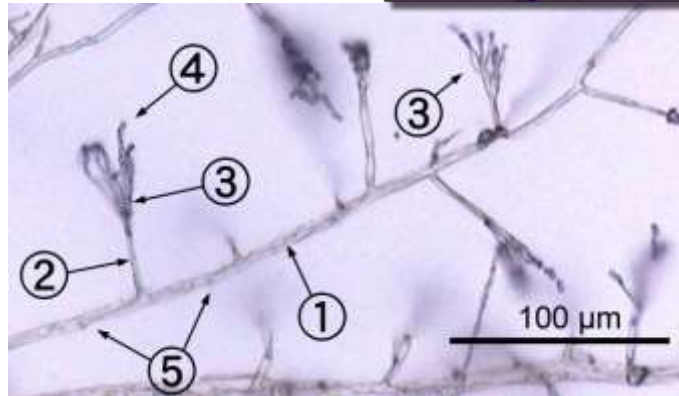
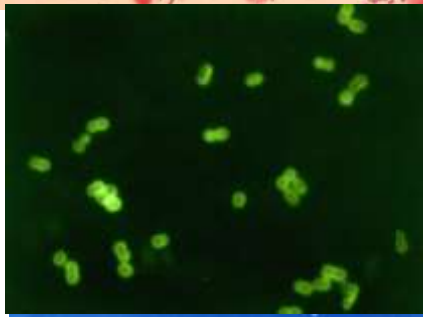
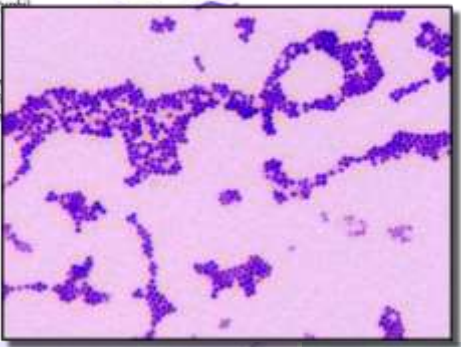
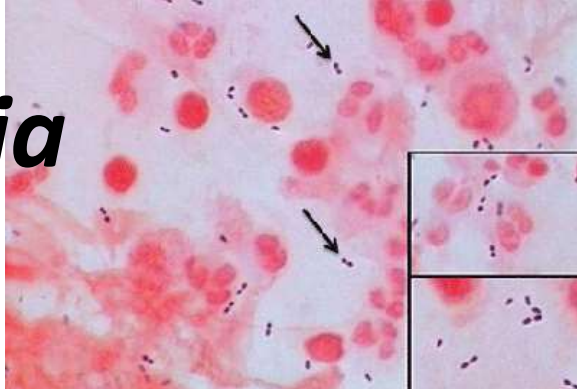


Microscopic Morphology

SHAPES OF BACTERIA



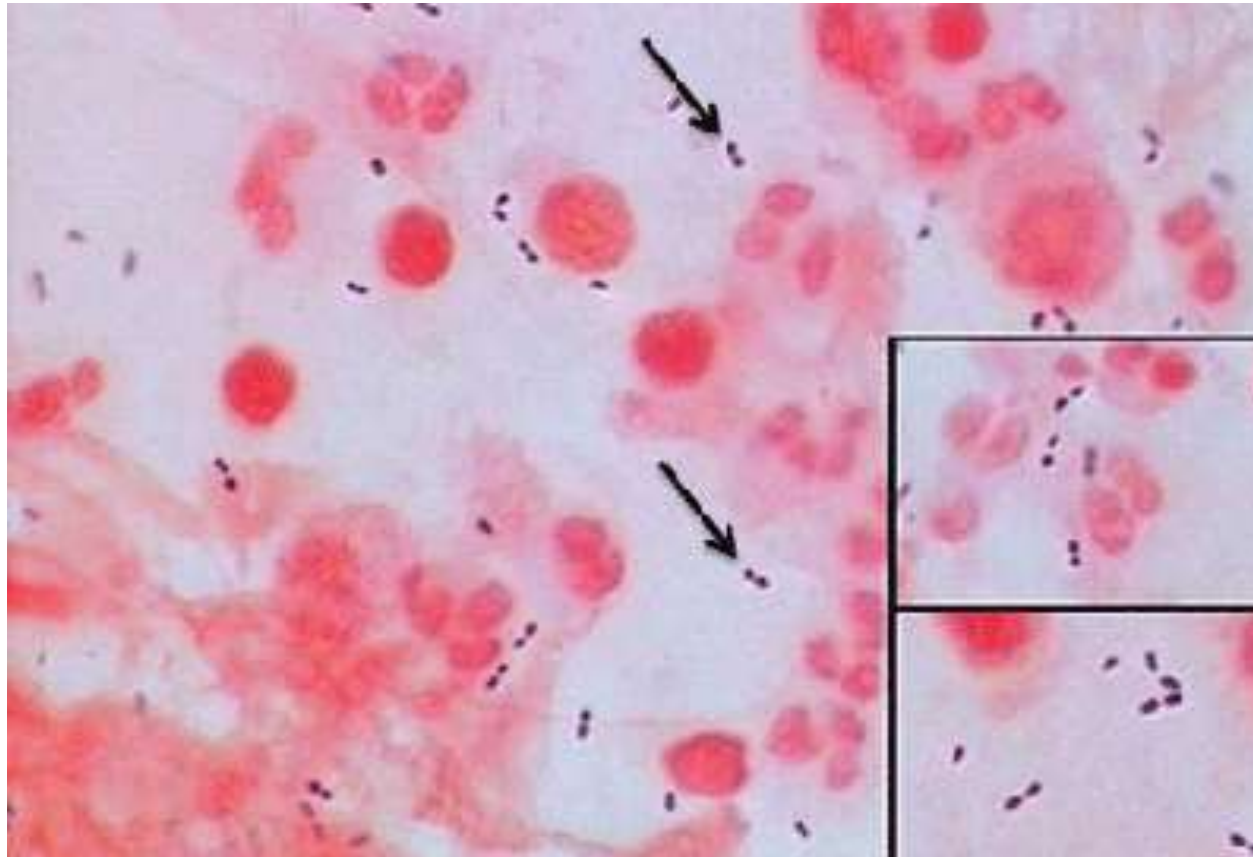
Bacteria



Fungi



Microscopic Morphology

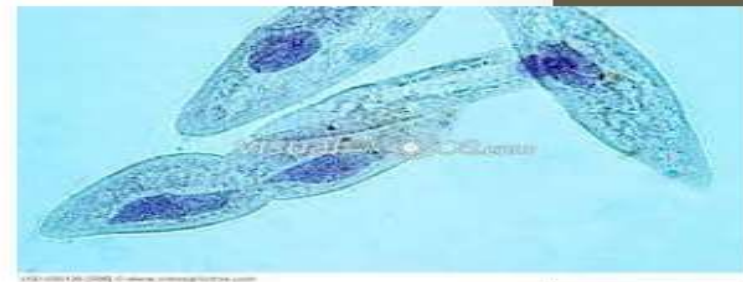
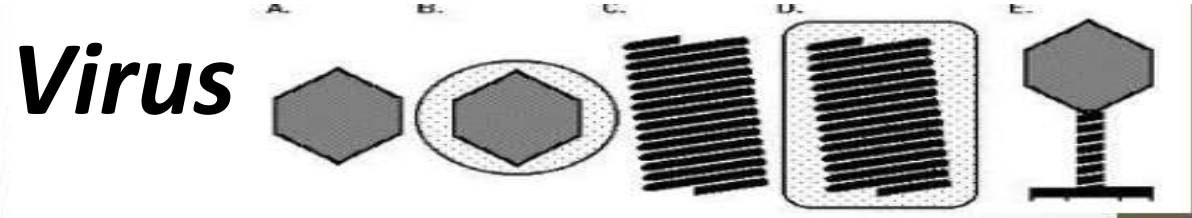


***Neisseria
gonorrhoeae***
In blood

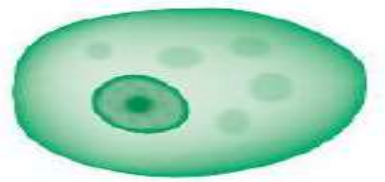
Microscopic Morphology



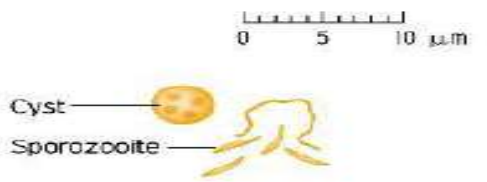
Transmission electron microscope image of influenza virus



Protozoa



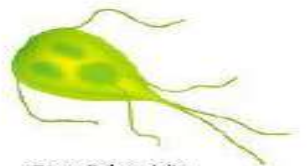
Entamoeba histolytica
 (Causes amoebic dysentery)



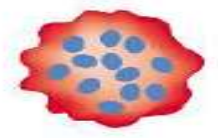
Cryptosporidium parvum
 (Causes cryptosporidiosis)



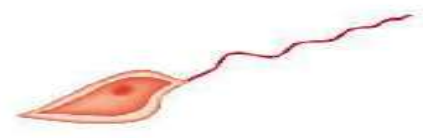
Trypanosoma brucei
 (Causes African sleeping sickness)



Giardia lamblia
 (Causes giardiasis)



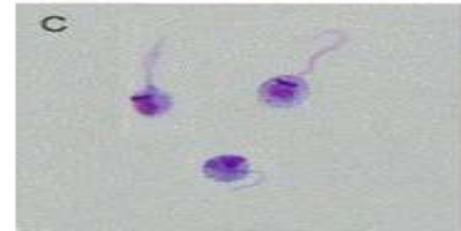
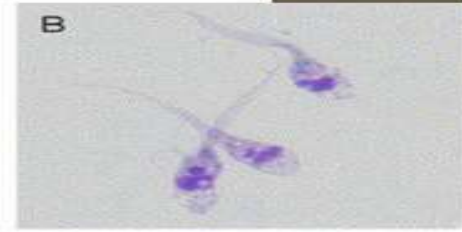
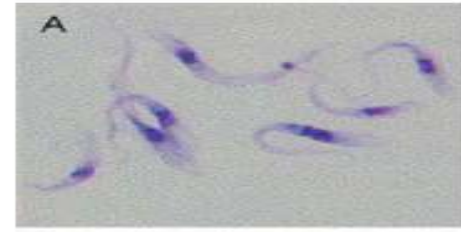
Red blood cell infected by *Plasmodium falciparum*
 (Causes malaria)



Leishmania major
 (Causes leishmaniasis)

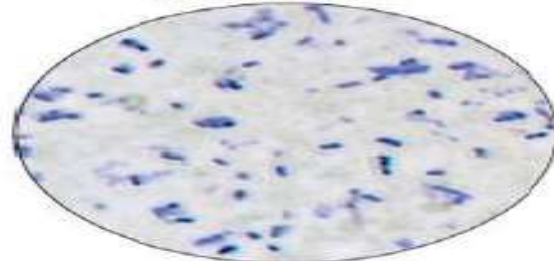


Toxoplasma gondii
 (Causes toxoplasmosis)



Microbe Stain

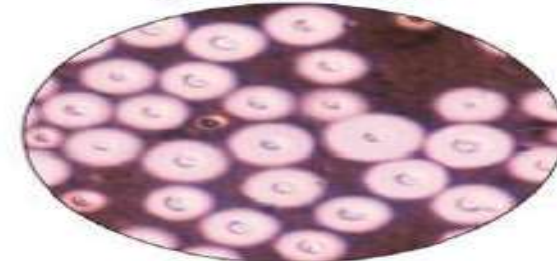
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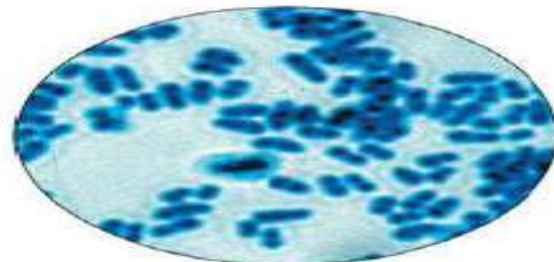
(a) Simple Stains
 Crystal violet
 stain of *Escherichia coli*
 (1,000×)



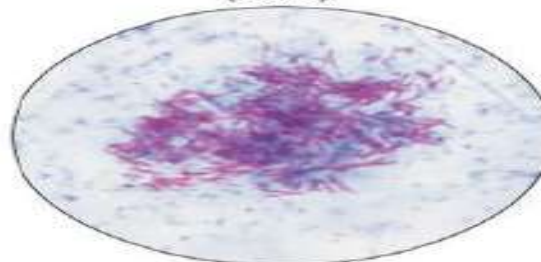
(b) Differential Stains
 Gram stain
 Purple cells are gram-positive.
 Red cells are gram-negative
 (900×).



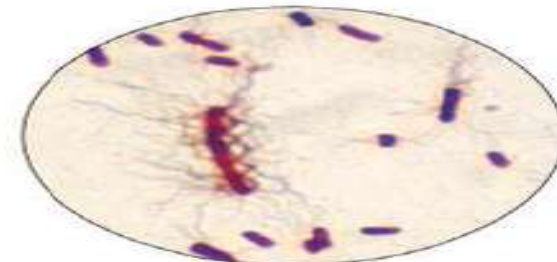
(c) Special Stains
 India ink capsule stain of
Cryptococcus neoformans
 (500×)



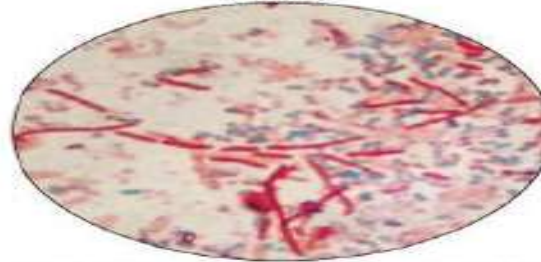
Methylene blue
 stain of *Corynebacterium*
 (1,000×)



Acid-fast stain
 Red cells are acid-fast.
 Blue cells are non-acid-fast
 (750×).



Flagellar stain of *Proteus vulgaris*.
 A basic stain was used to
 build up the flagella
 (1,500×).



Spore stain, showing spores (green)
 and vegetative cells (red)
 (1,000×)

1st Home Work

- What is the Acid-Fast stain?
- What is the unique characteristic described by this stain?
- Give examples of Acid-Fast Microorganisms>