GPAT

Online classes

Pharmaceutical Microbiology and Biotechnology

(13th -16th June 2020)

Organized By

Andhra Pradesh state Govt. APSCHE JNTUA JNTUK

Day-1 (13-06-2020)

Presented By

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GPAT
Microbiology

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- **Syllabus**

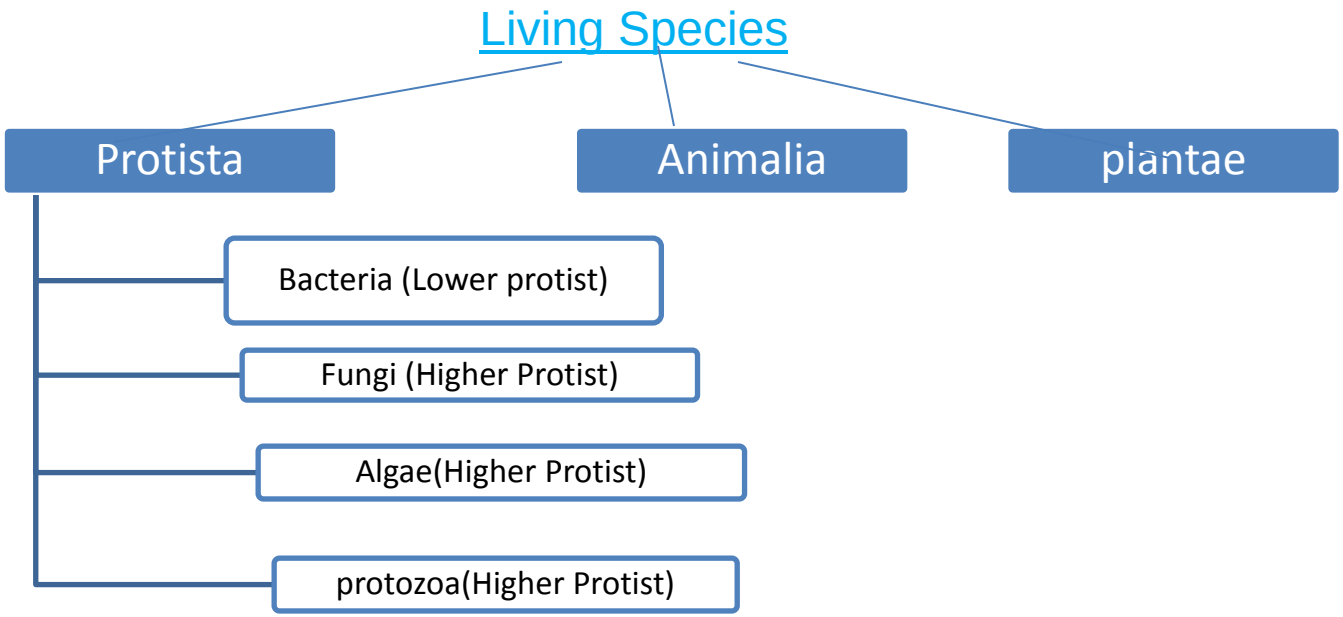
i. Introduction to Microbiology
ii. Microscopy and staining technique
iii. Biology of Microorganisms (Infections and causative organisms)
iv. Microbial spoilage
v. Vaccines & Sera
vi. Fungi and Viruses
vii. Aseptic Technique
viii. Sterilization & Disinfection
ix. Microbial Assay

Note:
Red- Most Important topics
Green-Important topics
blue- lesser than red and green
Black-Go through once

All four days (13th to 16th June 2020) PPT contain Questions from the previous GPAT exams. (1998-2019). Students can practice the old questions after preparing the everyday's slide.

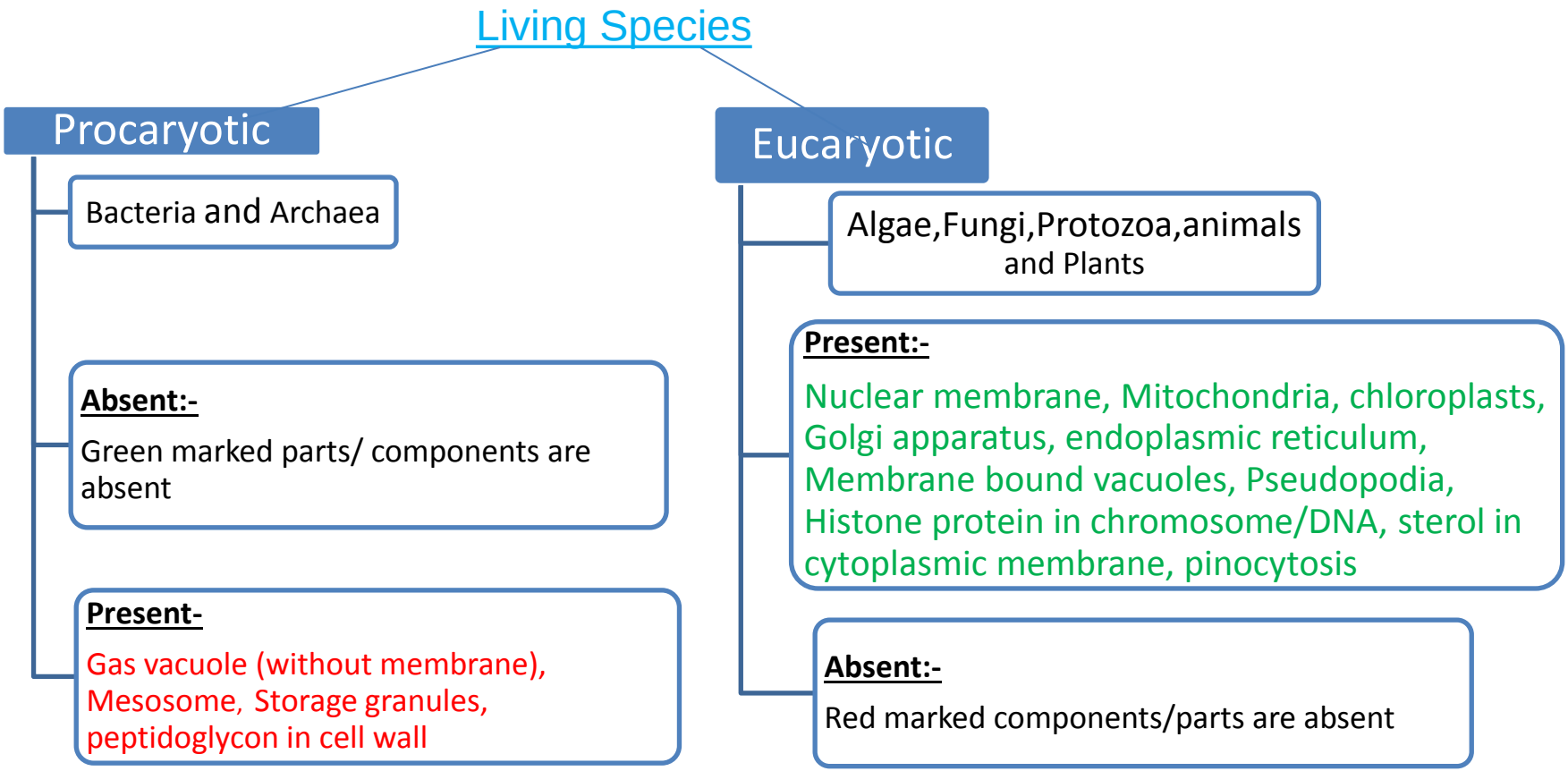
Introduction to Microbiology

E.H.Haekel - inclusion of Protista (microbes) apart from animalia and plantae



(Drawbacks- All microbes kept in same group protista
No clear differentiation made between bacteria, yeast, algae and fungali
Viruses were not included

Invention of electron Microscope-
presence of Nuclear membrane and well defined nucleus



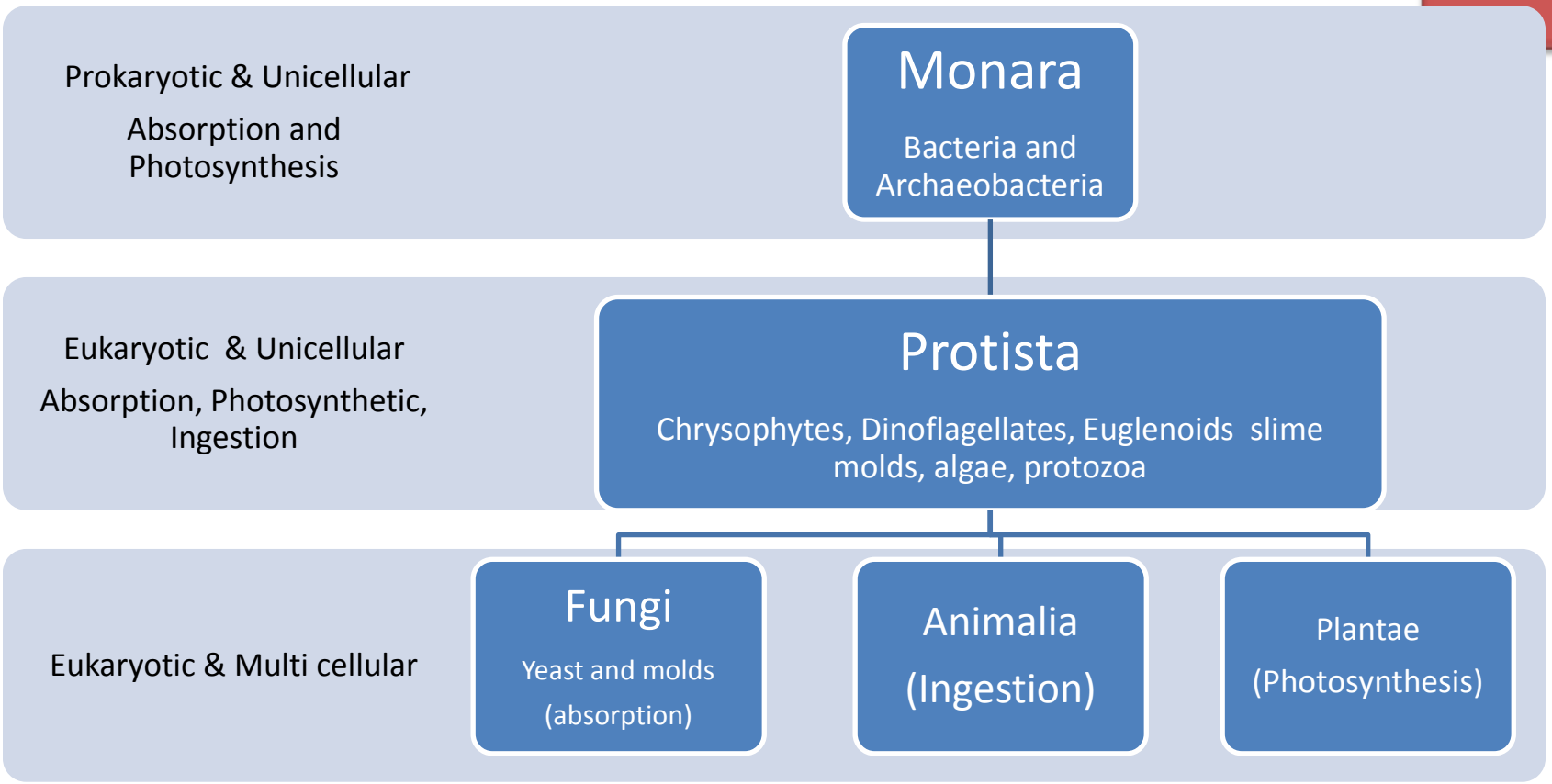
Other differences between Procaryotics and Eucaryotics

Property	Procaryotics	Eucaryotics
Size	1-5 µm	Greater than 5 µm
DNA location	Nucleoid region (without membrane)	Nucleus, (well defined Nucleor membrane) Mitochondria, chloroplast (membrane bound)
DNA/Chromosome	One circular Chromosome	More than one chromosome linear in nature
	No mitotic division	Mitotic nuclear division
	Haploid genome (only one gene copy) Genes are clustered	Diploid genome (more than one gene copy) Genes far away from each other
	Introns absent	Introns present
	23-70 % GC content	40% GC content
Transcription and translation	Transcription and translation occurs simultaneously in cytosol	Transcription in nucleus, translation in cytosol
Ribosome	70S present in cytoplasm	80S arrayed in ER membrane. 70S present in mitochondria and chloroplast
Cytoplasmic membrane	Sterols absent . But photosynthetic and respiratory bacteria contain sterols	Sterols present but wont do respiration and photosynthesis
Cell wall	Peptidoglycon	No peptidoglycon
Reproduction	Asexual (binary fission)	Asexual and sexual
Locomotor Organelles	Simple fibrils	Multiple fibrils with microtubles

Whittaker's Five kingdom concept

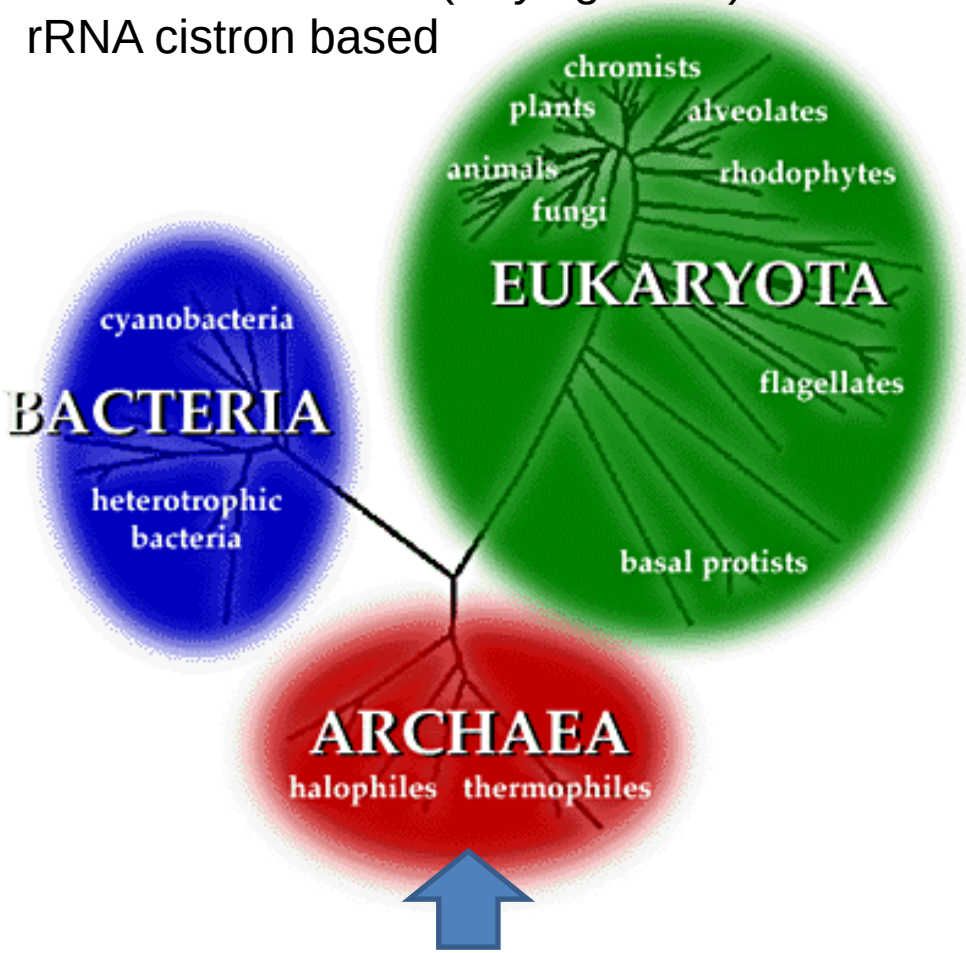
Based on

1. **Food intake (absorption, Photosynthesis, ingestion)**
2. **Unicellular/multicellular**
3. **Prokaryotic/Eukaryotic**



Note: microalgae photosynthesis, protozoa ingestion, some protista absorption some other protista overlap of photosynthesis and ingestion

Carl woese-Three domains of life (Phylogenetic) rRNA cistron based



LUCA-Last Universal Common Ancestor

Source- https://www.biology.iupui.edu/biocourses/N100/2k23domain.html

Property	BACTERIA	ARCHAEA	EUKARYOTA
Cell type	Prokaryotic		Eukaryotic
Cell size	Less than 5 micron	Less than 5 micron	More than 5 micron
Cell wall	Made of peptidoglycon	Does not contain peptidoglycon	Varies. In plants and fungi, composed of polysaccharides
Sensitivity to antibiotics	Yes	No	No
First amino acid during protein synthesis	Formylmethionine	Methionine	Methionine
DNA	Mostly circular chromosome and plasmids	Circular chromosome and plasmids	Linear chromosome,
Histones	No	Yes	Yes
Organelles	No	No	Yes
Ribosomes	70S	70S	80S
Membrane lipids	Ester linked unbranched fatty acids	Ether linked branched fatty acids	Ester linked unbranched fatty acids
High temperature	Can not Tolerate and grow (except some)	Tolerate and grow	Can not tolerate and grow
RNA polymerase	One type	Several types	Several types
Nuclear membrane	Absent	Absent	Present

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Five major groups of micro-organisms:

i.Bacteria

They are single celled disease-causing micro-organisms. They can be cocci, spiral or rod- shaped.

Grow in laboratory artificial media

ii. Fungi

Yeast unicellular
Molds multicellular
both are disease causing microbes. Bread moulds are common examples of fungi.
Grow in laboratory artificial media

iii. Protozoa

They mainly include organisms such as Amoeba, Plasmodium, etc. They can be unicellular or multicellular.

Some grow in lab media

Some in Intracellular growth

iv. Virus

Viruses are disease-causing microbes that reproduce only inside the host organism.

Can not Grow in laboratory artificial media

Intracellular growth

v. Algae

They include multicellular, photosynthetic organisms such as Spirogyra, Chlamydomonas, etc.

Grow in aquatic environment

Microscopic Techniques

Light Microscopy

Uses light (approx 400-700 nm)

Lower magnification

Specimen preparation few minutes or an hour

Both live and dead microbes

Useful magnification of 500x to 1500x

Low resolution

Inexpensive and requires low maintenance cost

Specimen observed under normal conditions

Electron Microscopy

electron beams (approx 1 nm)

Higher magnification

Specimen preparation takes several days

Only dead and the dried

magnification as high as 16000x to 1000000x

High resolution

Expensive and high maintenance

Specimen observed under vacuum

Light Microscopy Types

Bright field
Dead cells
Stained or unstained.
Gross morphological studies.
(Transmitted Light to generate image)

Dark field
Dead cells and rarely live cells
Unstained.
Motility studies
Gross morphological studies
(Diffracted Light to generate image)

Fluorescence
Fluorescent organism
Dead cells
Diagnostic techniques
(Emitted Light to generate Image)

Phase contrast.
Unstained
Extremely valuable for Live cells
(Refracted Light to generate Image)

Electron Microscopy Techniques

Transmission Electron Microscopy

Primary electron used to create images of 2D structures

Shadow casting

Thin layer of metal deposit over object (platinum metal)
Shadow of organism observed

Ultra thin sectioning

Cells embedded in plastic like material
60µm slices made

Freeze etching

Avoids chemical treatment
Frozen blocks of microbes cut into 60µm slices

Negative staining

Object coat with Phosphotungstic acid.
(Virus, Flagella and pili observation)

Scanning electron microscopy

Scattered secondary electrons and other scattered radiations used to create 3D structure. Lesser resolution than transmission electron microscopy

Others

1. Localization of cell constituents
2. Localization of enzymes
3. Autoradiography (radioisotopes0

Staining Techniques
Stains

Basic stains → Methylene blue, crystal violet, malachite green, basic carbol fuschsin, carbolfuschsin, safranin

Cationic (positive charge)

Acidic stains → Eosine, Acidic carbol fuchsin, rose bengal, Congo red, Indian Ink, Nigrosin,.

Anionic (negative charge)

Neutral Stains → Any complex salt of dye acid and dye base. Examples:-Eosinate of methylene blue. Giesma stain (Rickettes)

(no charge)

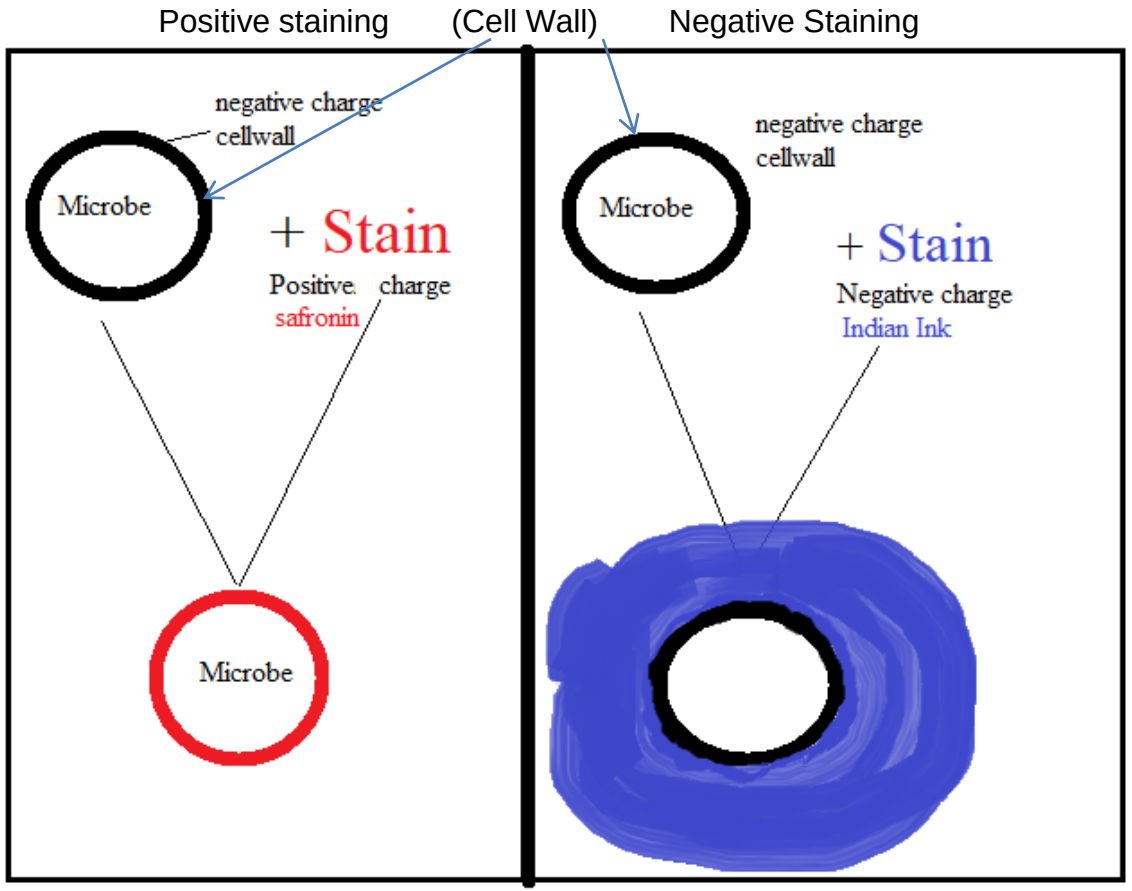
Staining Methods

1. Simple staining (One Dye)
2. Differential staining (more than One Dye)

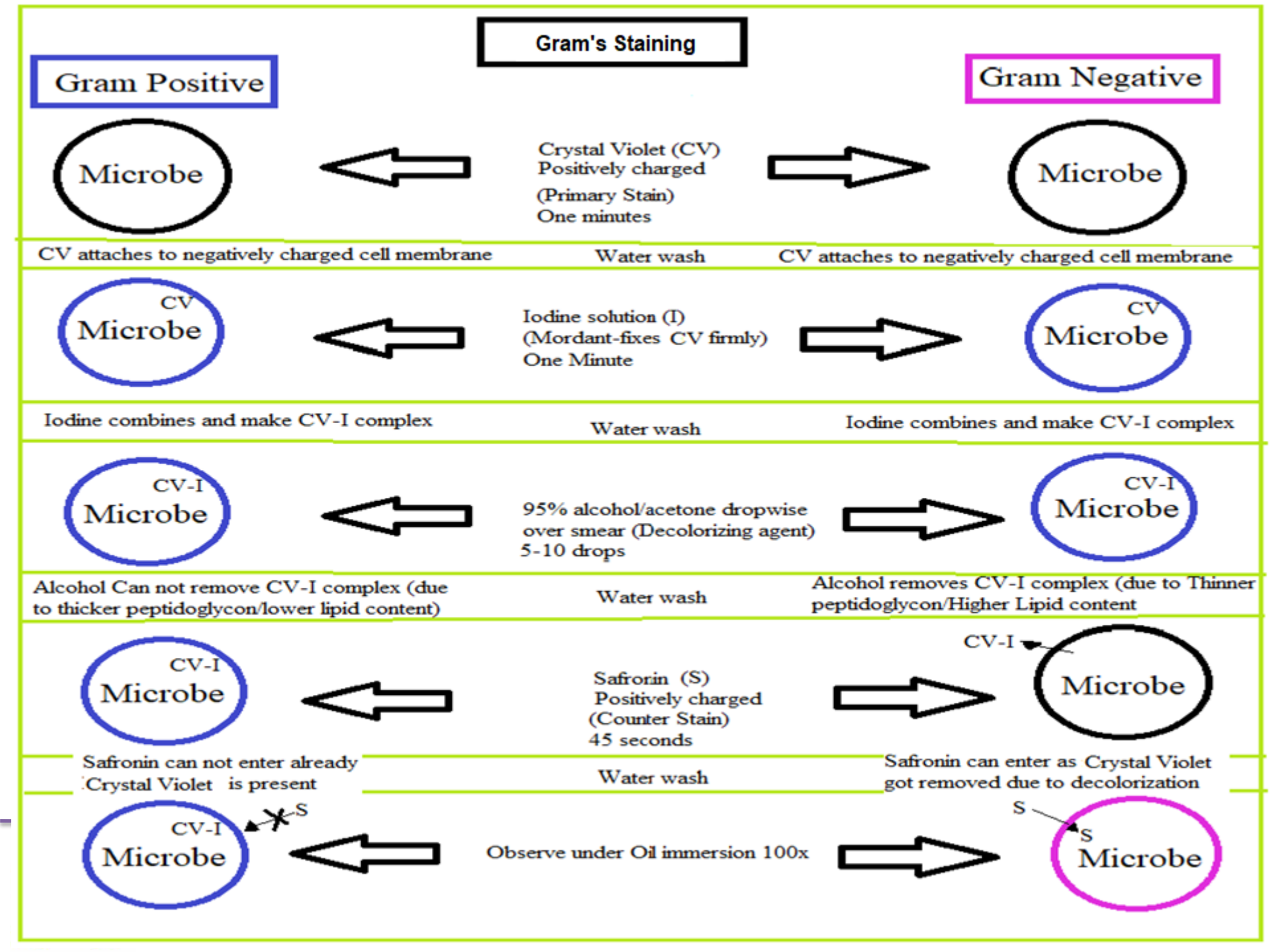
1.Simple staining

Simple staining:- Imparting color to the parts of microbes for morphological studies

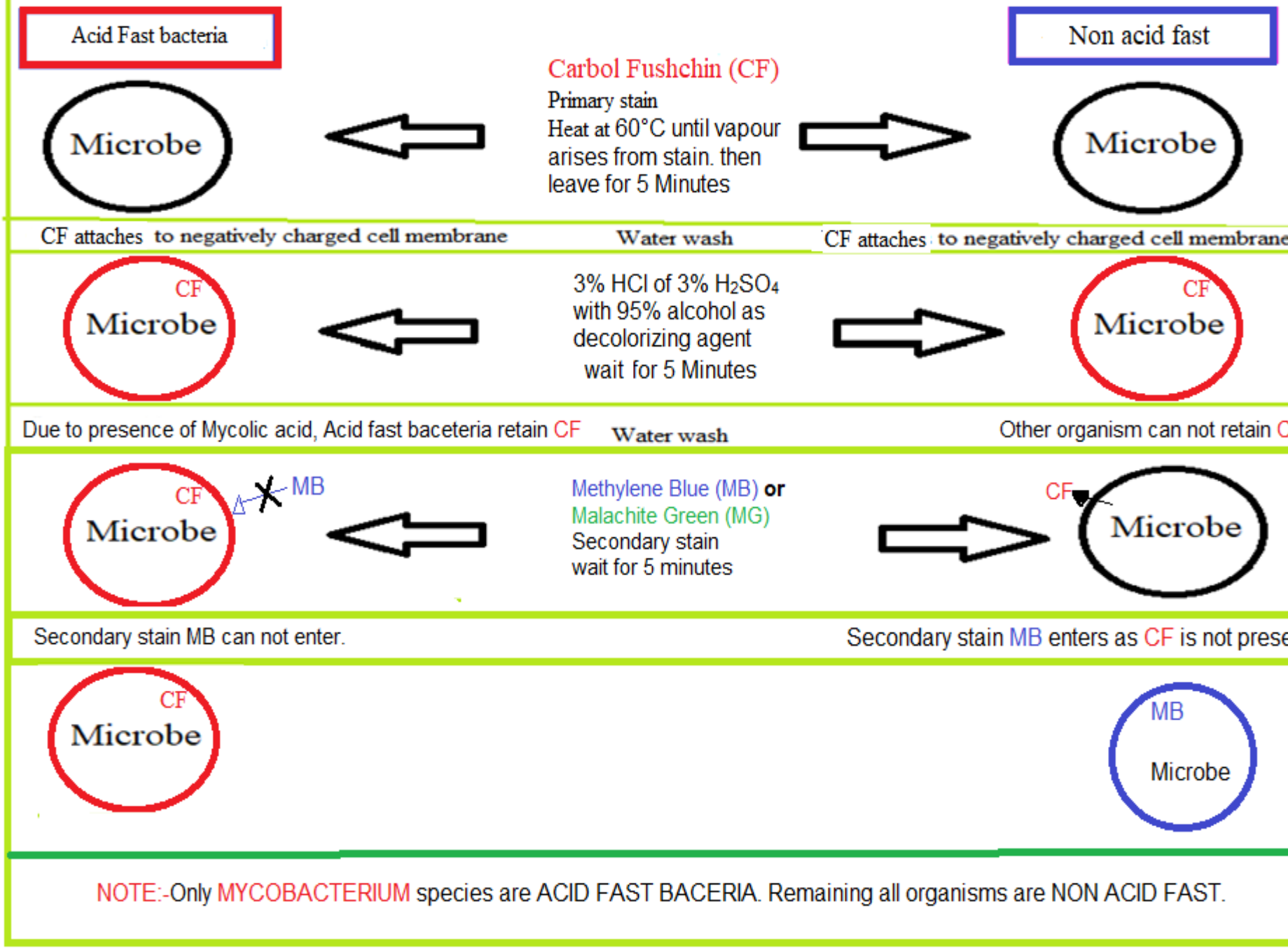
Differential staining:-
Differentiate the organisms (Gram's staining, Acid fast staining)
Or
Differentiate the parts of organisms



Gram's Staining



Acid Fast Staining/zeihl-Neelsen Staining



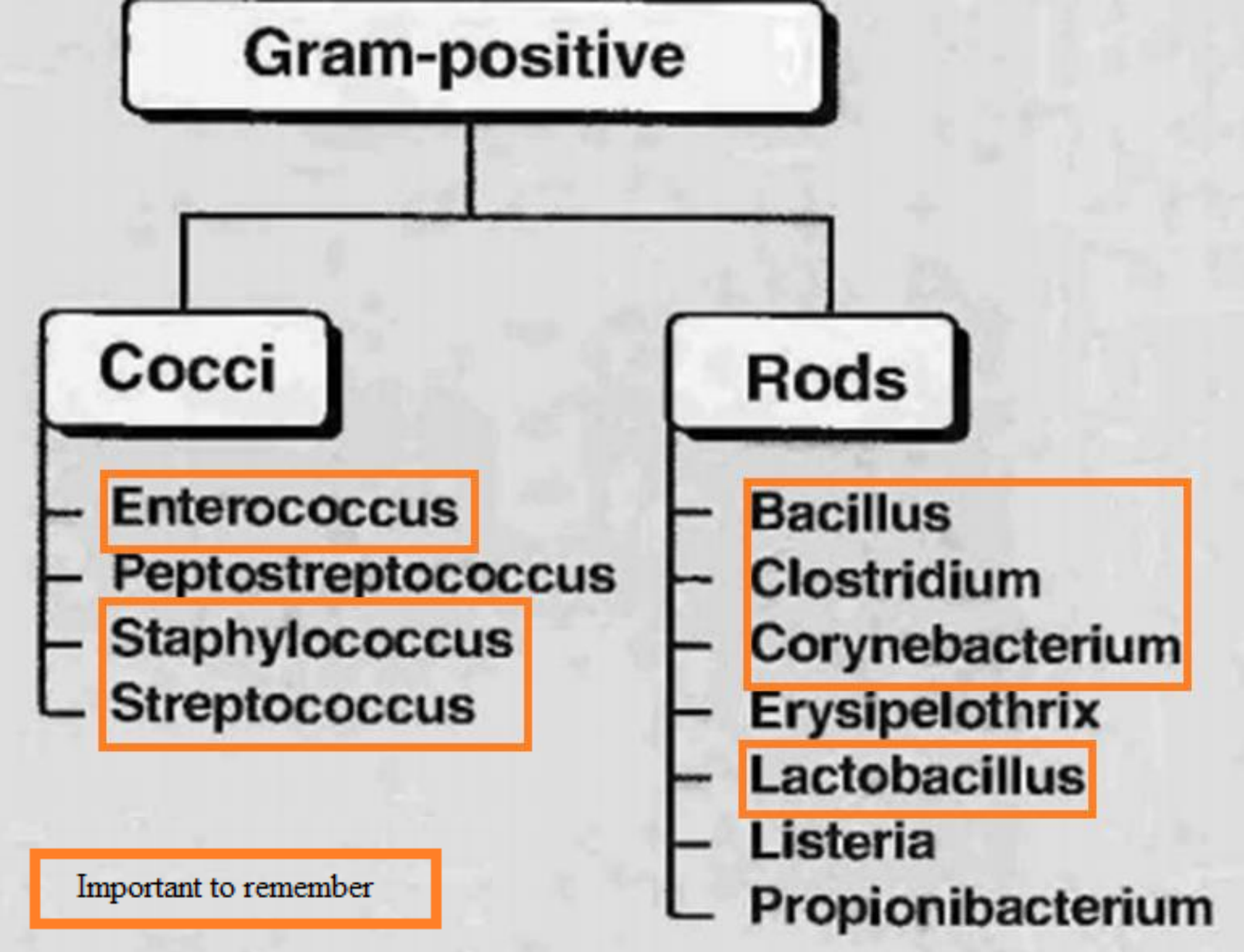
Differential Staining To differentiate the parts of MICROBES

1. Endospore staining (Schaeffer-Fulton Method and Dorner Method)
2. Capsule Staining
3. Flagella Staining (Liefson Stain)
4. Storage granules staining

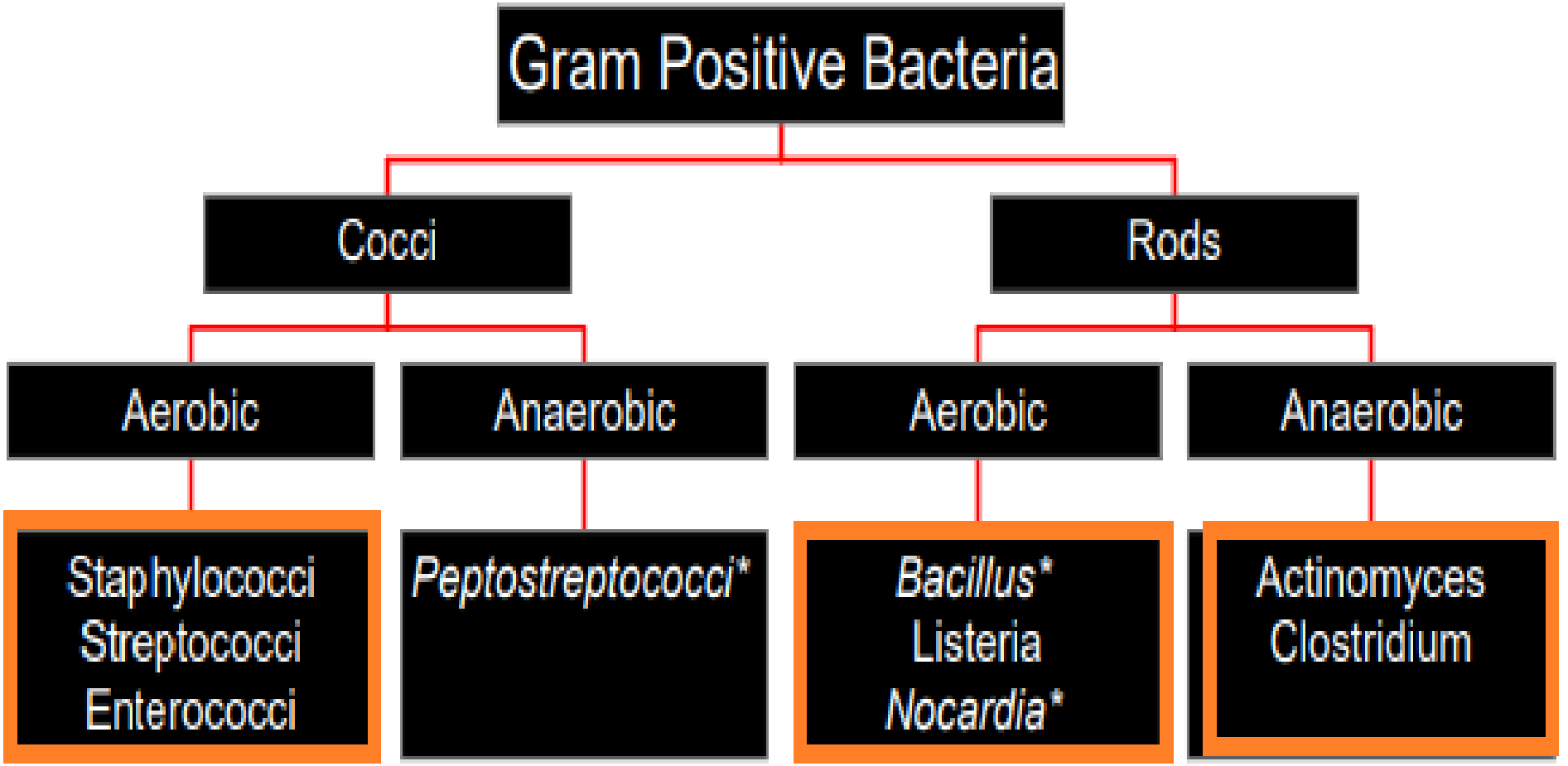
    Metachromatic Granule Staining (Albert's Staining)

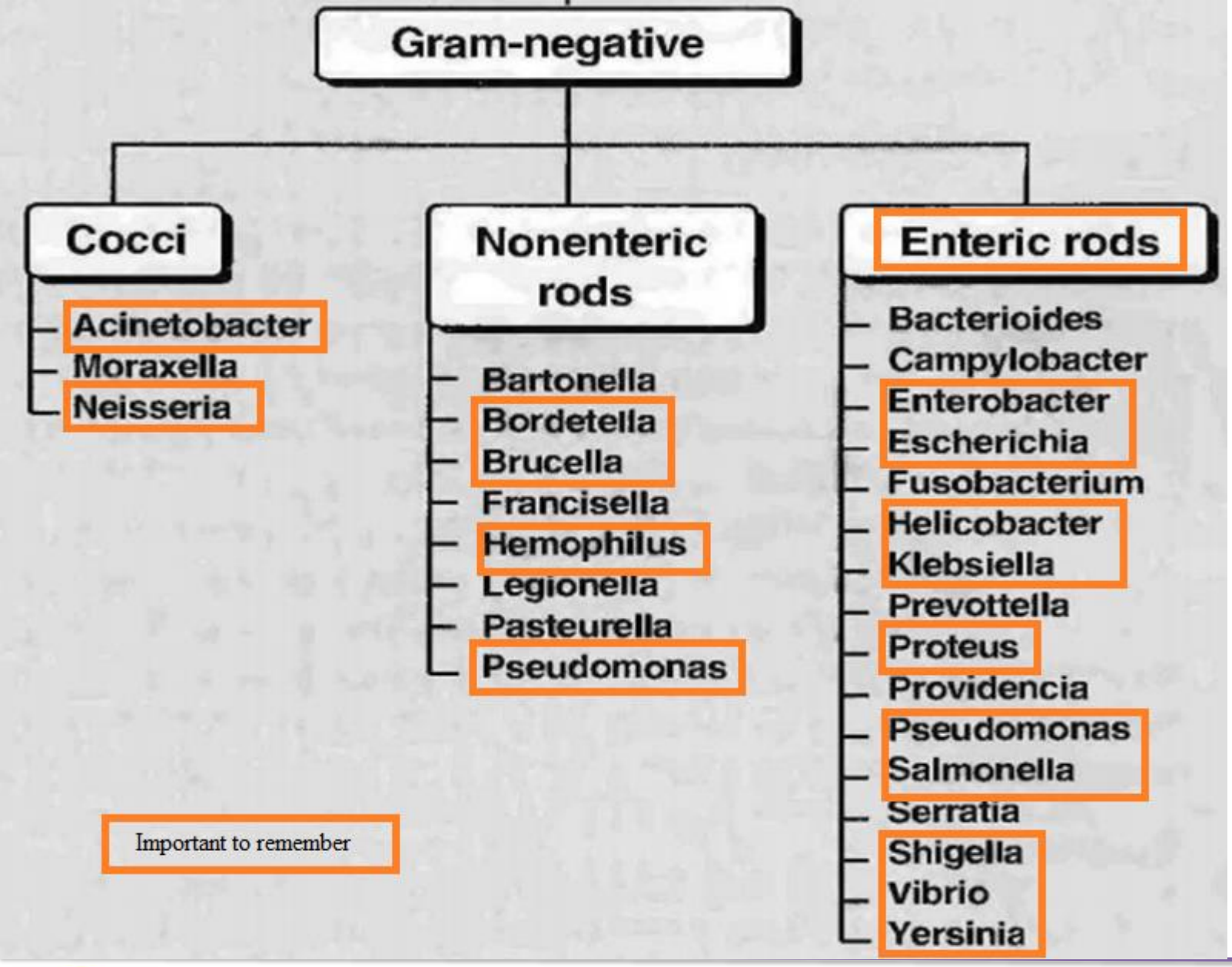
    Starch granules staining

    PHB granules staining

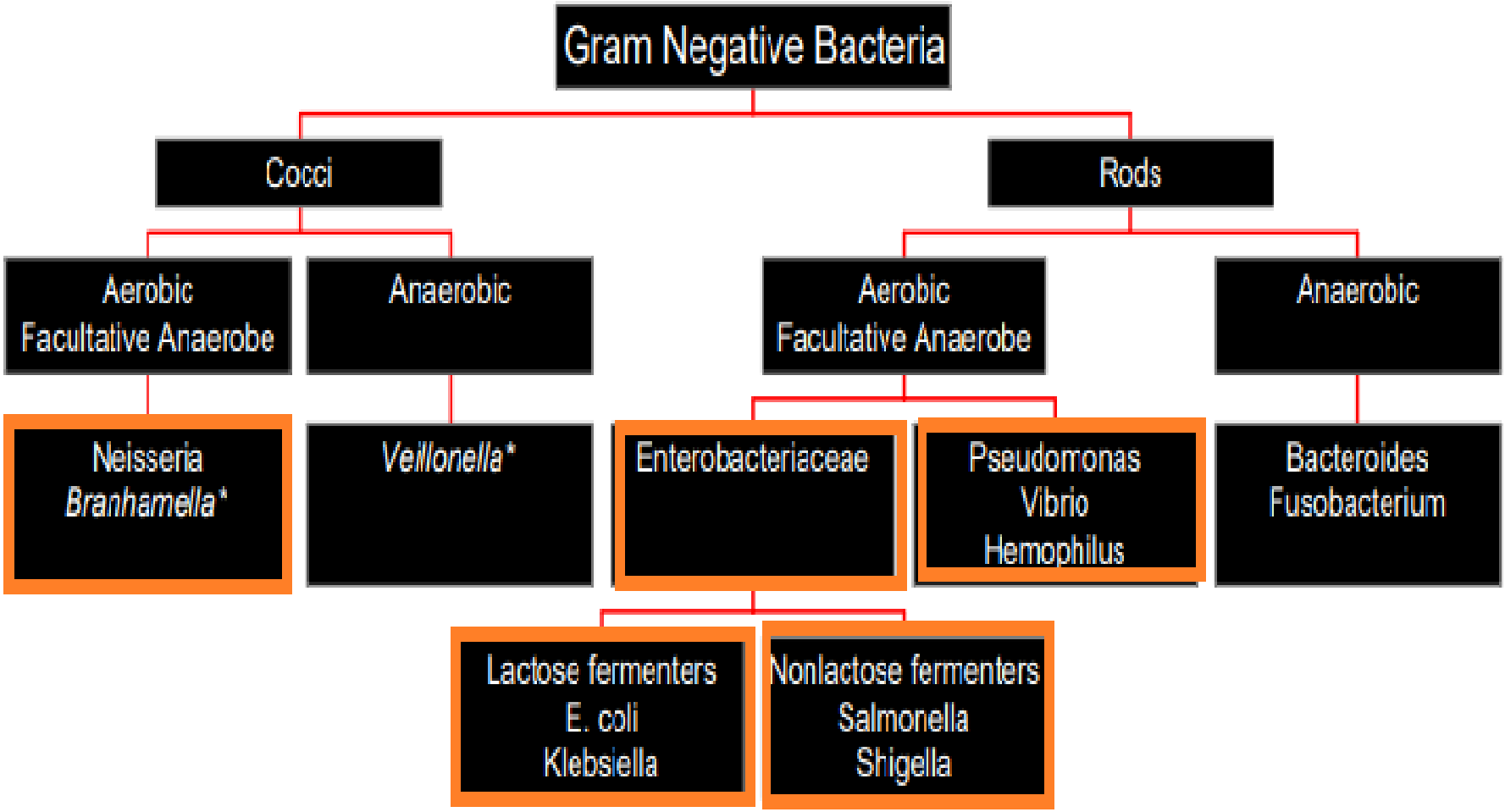

Gram-positive
Cocci
Enterococcus
Peptostreptococcus
Staphylococcus
Streptococcus
Rods
Bacillus
Clostridium
Corynebacterium
Erysipelothrix
Lactobacillus
Listeria
Propionibacterium
Important to remember

Diseases Point of view to remember

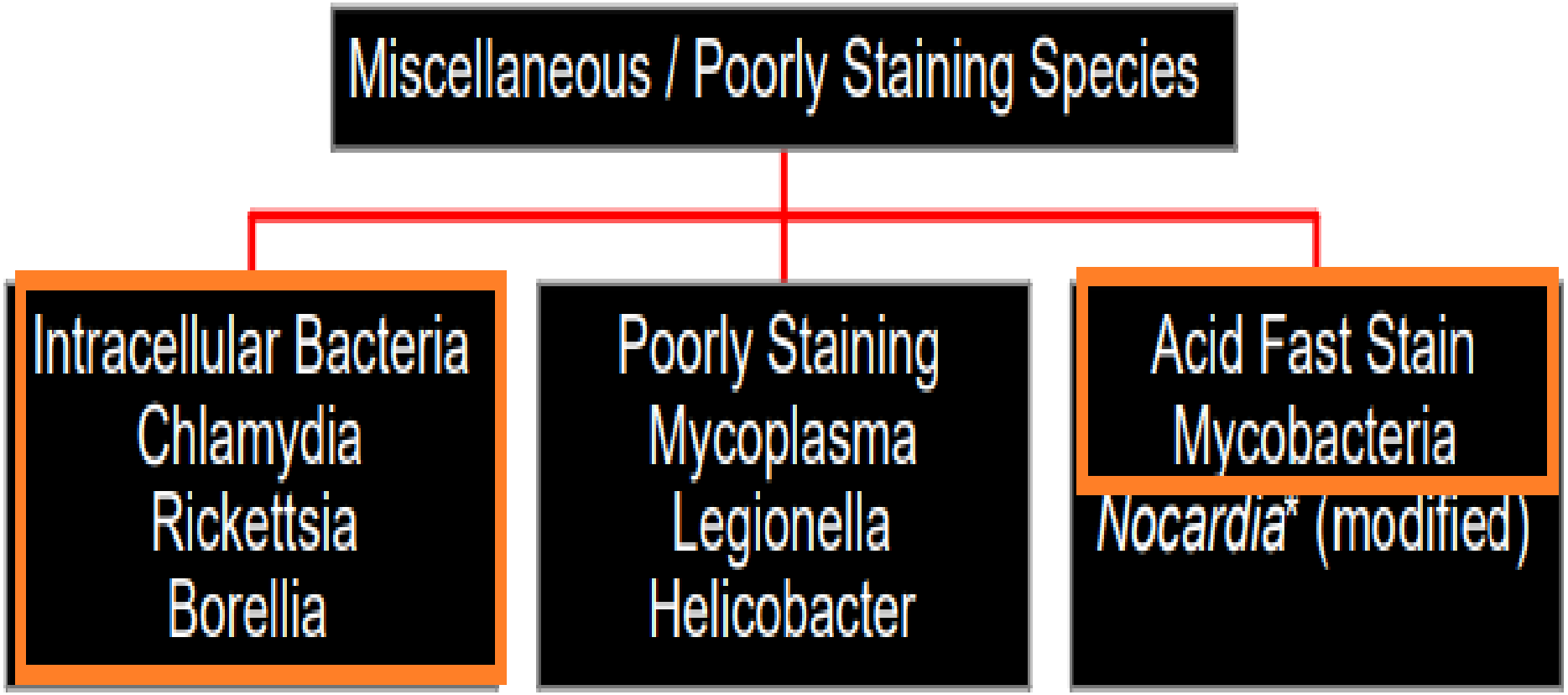



Gram-negative
Cocci
Acinetobacter
Moraxella
Neisseria
Nonenteric rods
Bartonella
Bordetella
Brucella
Francisella
Hemophilus
Legionella
Pasteurella
Pseudomonas
Enteric rods
Bacterioides
Campylobacter
Enterobacter
Escherichia
Fusobacterium
Helicobacter
Klebsiella
Prevottella
Proteus
Providencia
Pseudomonas
Salmonella
Serratia
Shigella
Vibrio
Yersinia
Important to remember

Diseases Point of view to remember



Diseases Point of view to remember



1. The Phase contract microscopy is valuable in studying living cells which are
   a) Stained
   b) Treated with fluorescent antibody
   c) Unstained
   d) Treated with fluorescent dye

2. Which of the following are obligatory intracellular parasites?

   a. Virus
   b. Fungus
   c. Mycobacterium
   d. Rickettes

A. All B. a, b and c C. c and d D. a and d

3.The major differences between the prokaryotic and eukaryotic protein synthesis mechanisms are in which part of the process?
   A. In the initiation of synthesis
   B. The chain termination process
   C. In the chain elongation process
   D. None of the above

4. Which of the following pairs is mismatched?

A) Aerobic, helical bacteria - gram-negative
C) Mycobacteria - acid-fast
B) Enterics - gram-negative
D) Pseudomonas - gram-positive

5. All of the following are gram-negative rods EXCEPT

A) Clostridium
B) Escherichia
C) Salmonella
D) Shigella

6. A gram'negative diplococcus associated with urinary tract infections, pelvic inflammatory disease, and Conjunctivitis, meningitis is:

A. N. Gonorrhoeae
B. Chlamydia trachomatis
C. Streptococcus pneumoniae
D. Hemophilus influenza

7.Safranin is used as a reagent to detect
- A. Gram negative bacteria
- B. Gram positive bacteria
- C. Acid fast bacteria
- D. Myxozoa

8. A specimen obtained from a patient's cerebrospinal fluid, cultured in specialized media for about five weeks showed the presence of bent rods and tested positive with ziehl neelsen reagent, Identify the organism.
- A. Niesseria meningitidis
- B. Mycobacterium tuberculosis
- C. Bacteroides fragilis
- D. Leptospira interrogans

Answers In the last slides of 16th June 2020 session

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Thank You