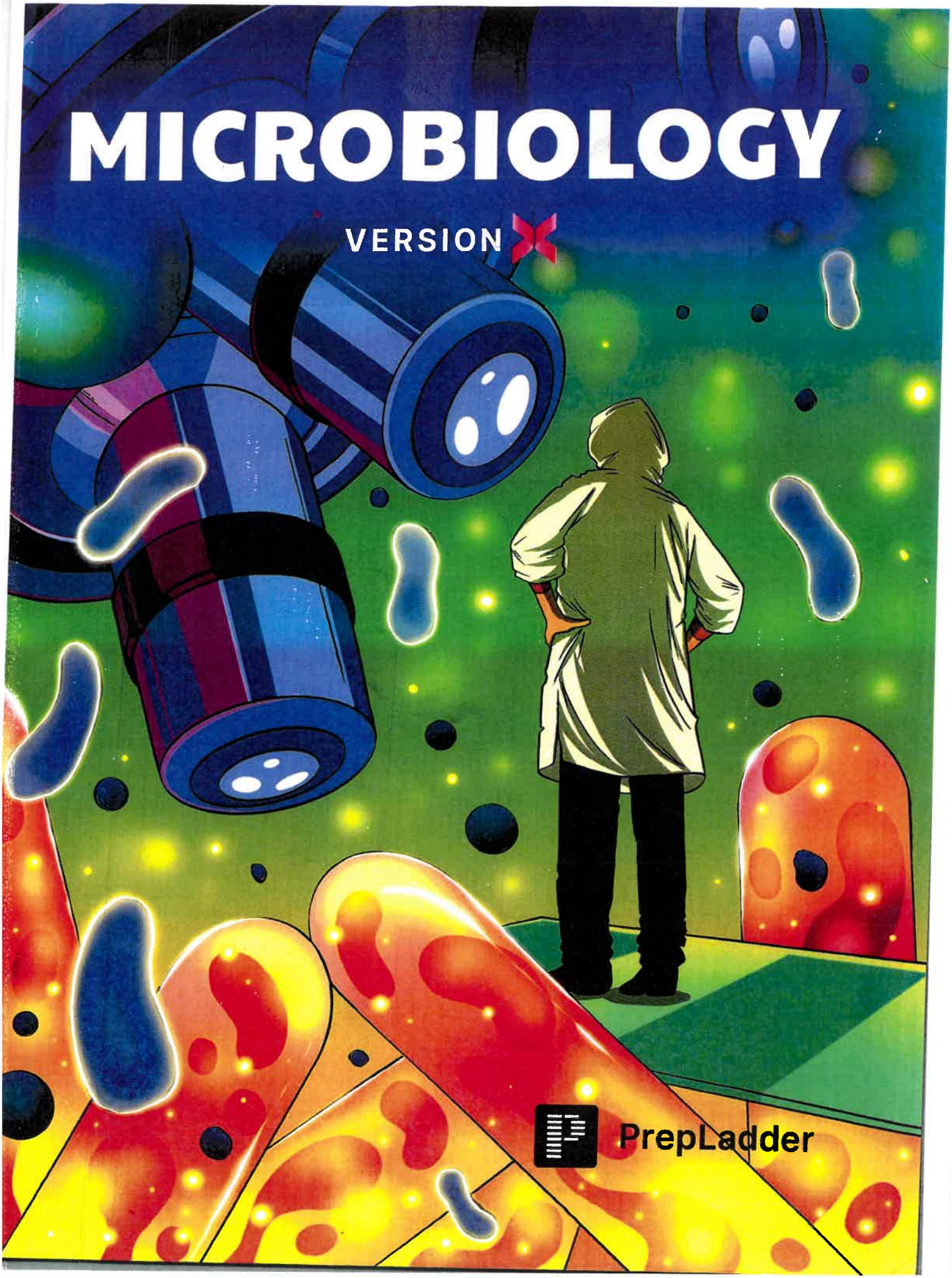
MICROBIOLOGY

VERSION X



PrepLadder

Structured Notes According to

MICROBIOLOGY

Revision friendly **Fully Colored Book/Structured Notes**

For Best results, watch the video lectures along with reading notes

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In case of any discrepancy between book and videos, Authors's videos on PrepLadder app should be considered.

CONTENTS

MICROBIOLOGY

VIROLOGY

MYCOLOGY

PARASITOLOGY

IMMUNOLOGY

MISCELLANEOUS

1. SCIENTISTS AND STAINS

SCIENTIST

00:00:12

LOUIS PASTEUR

- Pasteur institute of Paris named after him
- Gave the process of pasteurization of milk
- Gave information about Liquid Media
- Father of Microbiology, nowadays called the Father of Modern Microbiology
- Gave the fermentation principle
- Autoclave
- Theory of abiogenesis - disapproved by him
- Approved Germ Cell Theory
- Discovered vaccines
  - Cholera vaccine
  - Anthrax vaccine
  - Rabies vaccine

MNEMONIC

Louis Pasteur
Father
Car Vaccines

ROBERT KOCH

- Koch's postulates
- Koch's bacillus (Mycobacterium tuberculosis) → Called the Father of Bacteriology
- Discovered the cholera-causing organism
- Solid culture media
- Use of aniline dyes for staining
- Hanging drop motility

00:04:45

KOCH POSTULATES (4+1)

- Constant association of causative organisms with diseases
- Isolation in culture media possible
- Culture growth inoculated in animals should produce the same lesion
- Re isolation from the experimental animals is possible
- When an antigen enters the human body, body should produce antibodies in blood or serum

KOCH'S POSTULATES- EXCEPTIONS

- Bacteria that do not follow the postulates (Mnemonic: L P G)
  - Mycobacterium leprae
    → Cannot be grown in culture media, can only be grown in an animal
  - Treponema pallidum: causing Syphilis
  - Gonococci: Causing Sexually Transmitted Diseases

PAUL EHRLICH

- Discovered Ehrlichia bacteria
- Father of chemotherapy
- Toxin-antitoxin standardization (Nobel Prize)
- The acid-fast stain was identified by Paul Ehrlich and later modified by Ziehl and Neelsen
  (Mnemonic: PAUL EHRLICH)

JOSEPH LISTER

- Father of antiseptic surgery, The first chemical he used for antiseptic purposes was carbolic acid
  (Mnemonic: JOSEPH LISTER)

ANTON VON LEEUWENHOEK

- Father of light microscopy as he developed the light microscope (simple microscope)
- Simple microscope → uniocular microscope
  - 1st organism seen by him under microscope- named animalcules

JANSSEN DUO (Zacharias and Hans Janssen)

- Given credit for developing the compound microscope, used today

ERNST RUSKA

- Father of electron microscope

EDWARD JENNER

- First vaccine- Smallpox
  - Prepared from cowpox
  - Smallpox eradicated in 1980

KARRY B MULLIS

- PCR- Polymerase Chain Reaction

SANGER

- Gold standard PCR used nowadays

HC GRAM

- Gram staining

KLEINBERGER

- L forms
  - Cell wall deficient forms

ALEXANDER FLEMING

- Penicillin

BARBARA Mc CLINTOCK

- Transposons (Jumping genes)

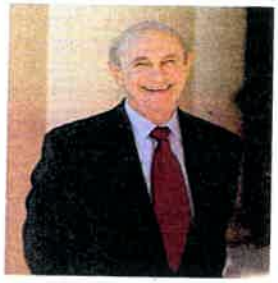
NOBEL PRIZES

00:13:35

MICHAEL HOUGTON

EMMANUELLE CHARPENTIER

HARVEY J. ALTER



JENNIFER A .DOUDNA

CHARLES M. RICE

- CRISPR Cas9- Genetic Editing
  - Gave the mechanism of CRISPR Cas9

- Gave the details of Hepatitis C virus → mechanism of what all this virus can do in the body

STAINS

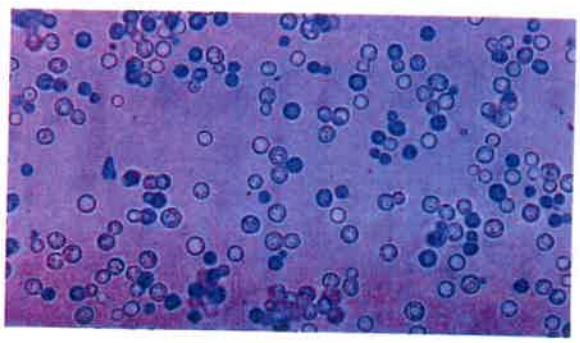
00:14:35

- Fixation
  - Heat fixation: passing the slide over a flame to fix the specimen
  - Chemical fixation
    → Methanol used

STAINING

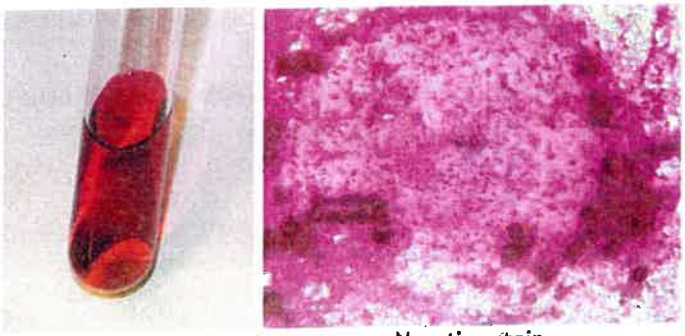
SIMPLE STAINS

- Methylene blue → gives blue color



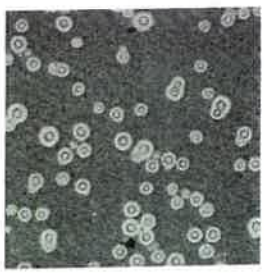
NEGATIVE STAINS

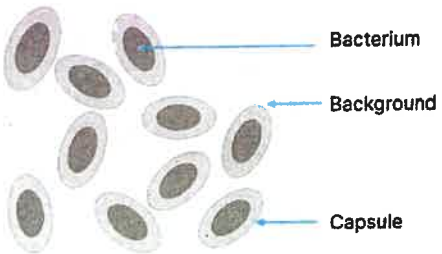
- Basic fuchsin → red color



Negative stain

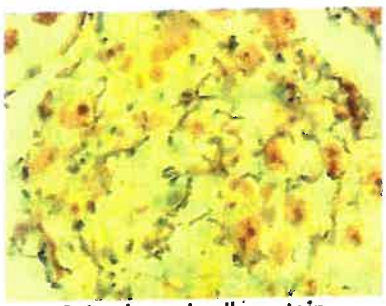
- India ink
- Nigrosine
- In negative stain
  - Background- stained
  - Capsule- unstained
  - Used to detect the fungus Cryptococcus neoformans





IMPREGNATION STAIN

- Silver stains
- Deposit stain on the surface of the object to make it look thicker
- Silver stains used in case of thin structure
  - Flagella
  - Spirochetes (causes Syphilis- Genital ulcers)



Spirochetes in silver stain

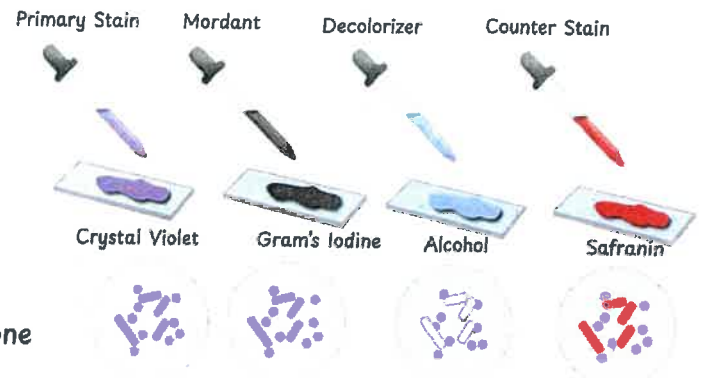
- Silver stains
  - Fontana stain for fluid specimen
  - Levaditi's stain for tissue specimen
- Warthin-Starry silver stain- stains black → used for bacterium H.pylori

DIFFERENTIAL STAINS

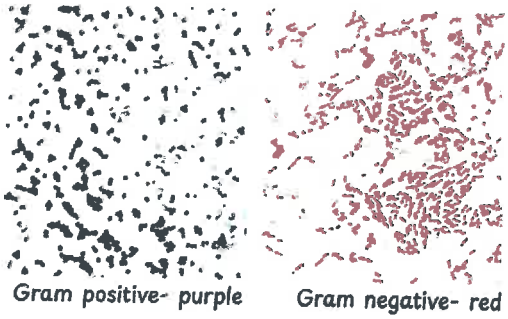
- Gram stain
  - To differentiate between Gram-positive and Gram-negative organism
- Acid fast stain
  - To differentiate whether organism is acid fast or not
  - Albert stain

GRAM STAIN

00:22:20



- 1st stain- Crystal violet → primary stain
  - Timing- 1 minute
  - Gram positive organism- purple
  - Gram negative- purple
- Mordant added -fixer → iodine
  - Timing- 1 minute
  - Gram positive organism- purple
  - Gram negative organism- purple
- Decolorization- decolorizer added → alcohol/ acetone
  - Timing- few seconds
  - Gram positive- purple
  - Gram negative- colorless
  - Most crucial step
- Secondary stain added- Safranin red
  - Timing- 1 minute
  - Gram positive organism- purple
  - Gram negative organism- reddish pinkish
- Final result as shown



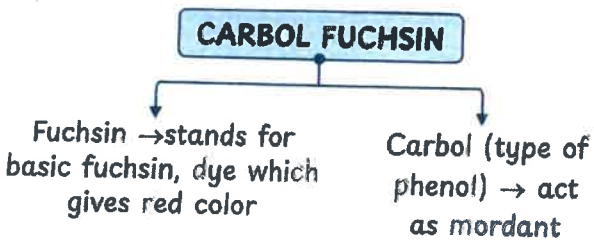
- Poorly Gram staining organism: cannot be grouped under any category
  - Mycoplasma (smallest bacteria) → very small so does not take the color
  - Rickettsia, Chlamydia } Small and intracellular
  - Spirochetes → thin and slender so does not take the color

PRINCIPLE OF GRAM STAINING

- Gram positive cell wall has more peptidoglycan and hence retains primary dye longer
- On adding decolorizer Gram negative becomes colorless as Gram negative cell wall has more lipopolysaccharide (lipid content) making it permeable to secondary dye → alcohol solubilizes lipid

ZIEHL NEELSEN STAIN (ACID FAST STAIN)

- 1st stain- Carbolfuchsin



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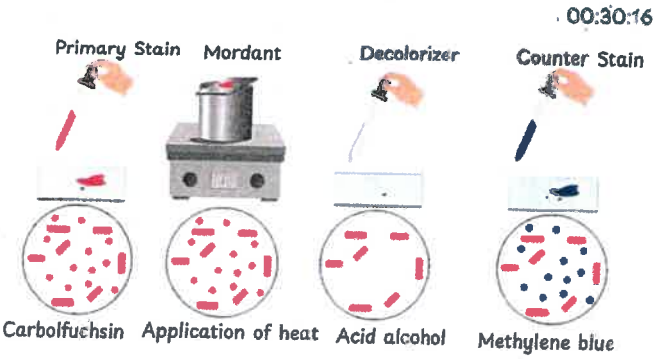
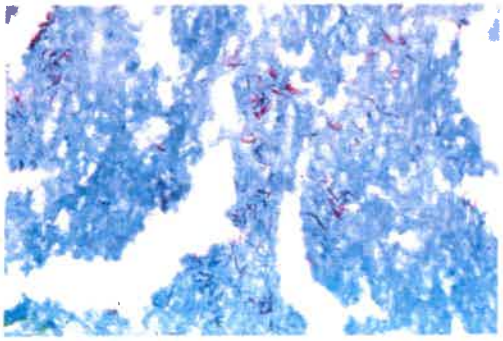


- To make sure Carbolfuchsin goes inside the bacterial wall → intermittent heating done
- Adding Carbol fuchsin + intermittent heating done for 5 minutes → imparts red color to organism
- Decolorization → mixture of acid -alcohol used → sulphuric acid
- Secondary stain- Methylene blue
- It is called an acid fast stain as → most commonly ZN staining in our country done for Tuberculosis and TB is acid fast
- Acid fast means that even when sulphuric acid is added TB not going to leave its color, meaning it is acid-resistant

Organism red Background blue



Z-N STAINING

- Purpose of heating
  - TB has lipids- Mycolic acid → like a barrier, it does not let any color go inside
  - for carbolic acid to enter inside → heating required
- Sulphuric acid percentages for different organisms to be acid fast

Mycobacterium tuberculosis	20-25% $H2SO_4$
Mycobacterium lepra, coccidian parasite	5% $H2SO_4$
Nocardia, Legionella	1% $H2SO_4$
Spores, head of sperm	0.25-0.5% $H2SO_4$

Important Information

- Fite Faracco Stain (in dermatology)- 5% H_2SO_4

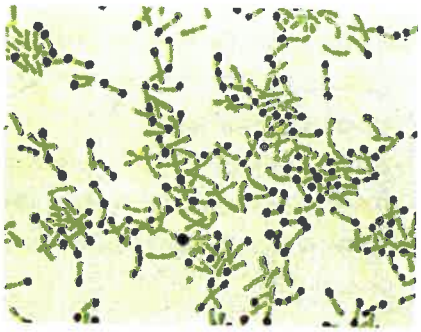
ACID FAST ORGANISMS

- Mycobacteria
  - TB
  - M. leprae
  - Atypical Mycobacteria
  - TB Vs Mycobacteria
    → TB is acid (20% sulphuric acid) and alcohol fast (95% alcohol)
    → Atypical mycobacteria is only acid fast
- Nocardia
- Legionella micdadei
- Isospora
- Cyclospora
- Cryptosporidium } Coccidian parasites
- Sperm heads
- Spores
- Hooklets of hydatid cyst
- Taenia saginata eggs

ZIEHL NEELSEN STAIN MODIFICATION

- Modification
  - Kinyoun stain also known as cold ZN stain/ Gabbet's stain
  - → No heating done
  - → Concentration of phenol is increased- acts as mordant
  - → Total 3 steps- add Carbol fuchsin → acid alcohol decolorizer → add methylene blue
  - → Used for detecting Coccidian parasites

ALBERT STAIN



- Specific to Corynebacterium diphtheriae → volutin granules
  - Two granules present at the poles known as the Volutin granules
- Albert solution 1- Toluidine Blue, Malachite Green, Glacial Acetic acid
- Albert solution 2- Iodine
- Due to malachite green – color of Corynebacterium diphtheria is green
- Due to Toluidine Blue – purple color of volutin granules
- Toluidine blue- metachromatic stain
- Sequence of Albert staining
  - Toluidine blue → iodine → Malachite green

STAINS FOR VOLUTIN GRANULES

- Ponder's stain
- Loeffler's methylene blue- best for volutin granules
- Albert stain
- Neisser stain

FLAGELLA STAINS

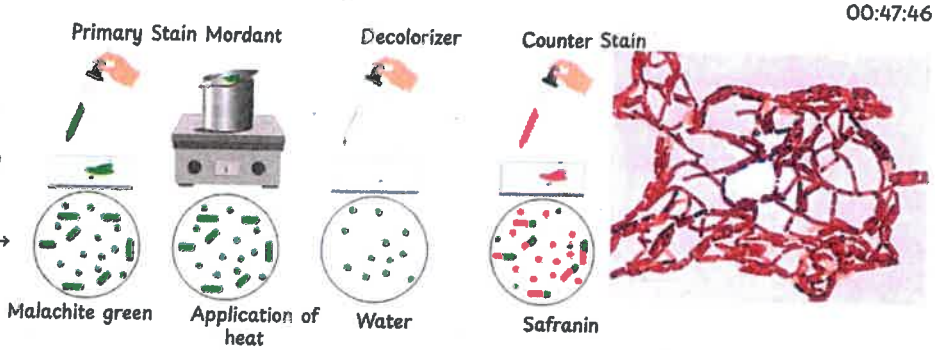
00:46:27

- Leifson and Ryu stain
  - Dye- Basic Fuchsin for flagella → red
  - Mordant – Tannic acid
  - Dye- Methylene Blue for cell → Blue

SPORE STAINS

00:47:46

- Spore- dormant stage of bacteria but still capable of causing infection
- Stained by Schaeffer and Fulton stain / Modified Ashby stain
- 1st stain- Malachite green stain→ gives green color
- 2nd stain- Safranin red
- Organism- red color
- Spore – green color



MCQ's

Q. In Gram-stain the mordant is?
a. Tannic acid
b. Loeffler's mordant
c. Lugol's iodine
d. Bovine's fixative

Ans. (c)

Q. After the primary stain and mordant has been added but before the decolorizing agent has been used, gram-positive organisms are stained....., and gram-negative organisms are stained.....
a. Purple, Purple
b. Purple, colorless
c. Purple, pink
d. Pink, pink

Ans. (a)

Q. Metachromatic granules stained by all except?
a. Albert stain
b. Neisser
c. Ponder
d. Kinyoun

Ans. (d)

Q. Silver Impregnation method of staining is used to demonstrate?
a. Mycobacteria
b. Spirochaetes
c. Both of the above
d. None of the above

Ans. (b)

Q. Which of the following is acid-fast with 20% H_2SO_4 ?
a. M. avium
b. M. Leprae
c. Actinomyces
d. Nocardia
Ans. (a)

Q. In the ZN staining procedure, the secondary stain is?

a. Crystal violet
b. Safranin
c. Methylene blue
d. Alcohol

Ans. (c)

Q. Correct order of gram staining?

a. Carbol fuchsin- Iodine- Acetone- Methyl violet
b. Crystal violet- Iodine- Acetone- Safranin
c. Methyl violet- Acetone- Iodine- Safranin
d. Crystal violet-Carbol fuchsin-Acetone- Iodine

Ans. (b)

2. MICROSCOPES

FEATURES OF MICROSCOPES

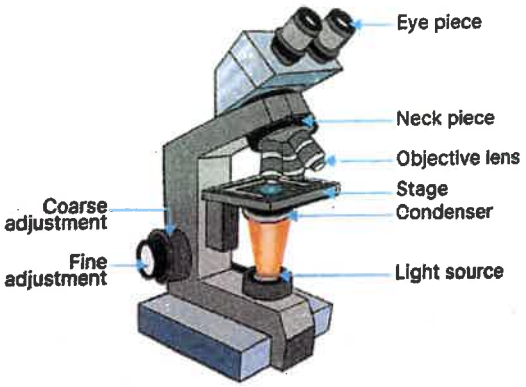
00:00:13

- Magnification
  - Lens
- Resolution: Ability to differentiate two points as separate
  - Eye: 0.2mm
  - Light Microscope: 0.2 microns
  - Electron Microscope: 0.2-0.5 nm
    → Viruses only seen under electron microscope
- Contrast
  - Dyes

LIGHT MICROSCOPE

00:02:34

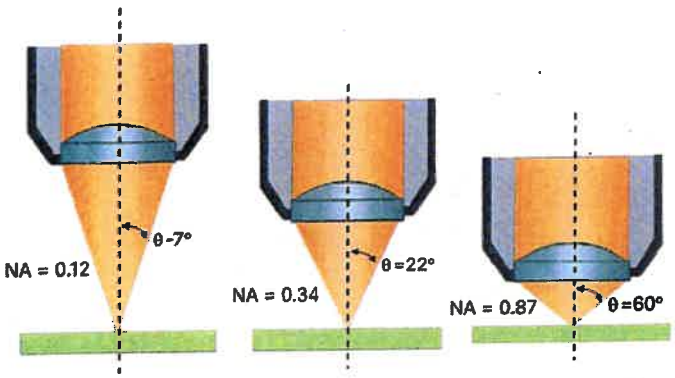
- Source of light: transmitted light
- Slides: kept on the stage
- Condenser
  - Location- below the stage
  - Condenses the light onto the slide
  - Diaphragm is present inside the condenser to adjust the amount of light entering
- Two lenses
  - Objective lens- near slide/ object
  - Eyepiece- near eye
- Lens revolve around the neck piece
- Two knobs
  - Big knob- Coarse adjustment
  - Small knob- Fine adjustment



NUMERICAL APERTURE

00:08:02

- When light is passing through, an angle between the lens and light created → angle divided in half → half the angle known as θ → as the magnification of lens ↑→ the value of θ also ↑
- Numerical aperture (NA) = n (sin θ) where n= refractive index, θ= half the angle
- Lens magnification ↑→ value of θ ↑→ numerical aperture ↑
- As the lens size/ magnification ↑→ numerical aperture ↑



MAGNIFICATION OF LENS

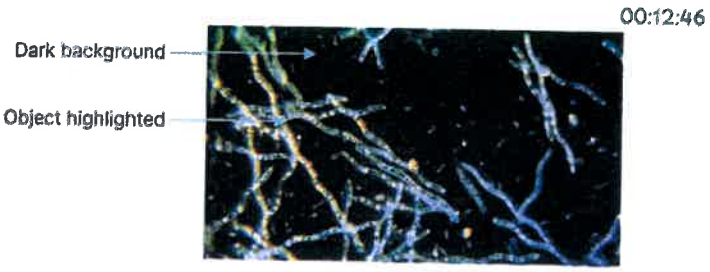
- Eyepiece lens
  - Only one lens and there is no variation, magnification is always 10X
- Objective lens
  - Scanner: 4X
  - Low power: 10X
  - High power: 40X
  - Oil immersion: 100X
- Total magnification = objective x eyepiece

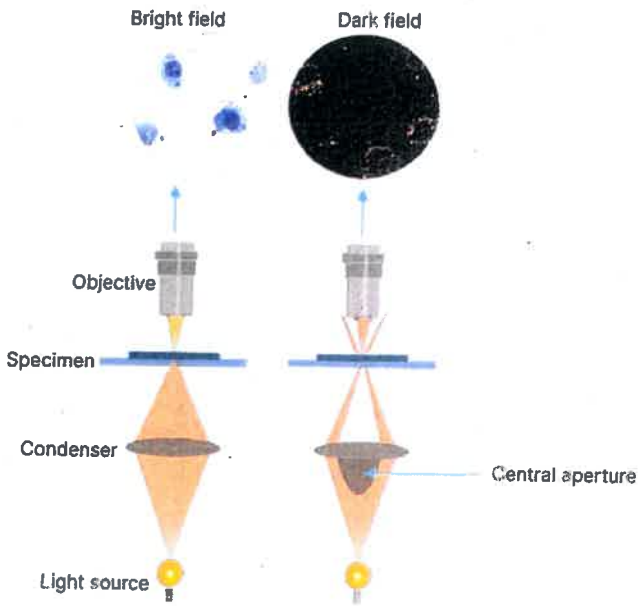
OBJECTIVE LENS	MAGNIFICATION	EYEPIECE	TOTAL MAGNIFICATION
SCANNER	4X	10X	40X
LOW POWER	10X	10X	100X
HIGH POWER	40X	10X	400X
OIL IMMERSION	100X	10X	1000X

- Maximum magnification of light microscope = 1000X
- Maximum magnification of objective lens = 100X

DARK FIELD MICROSCOPE

- Reflected light which illuminates the object
- Used for thin structures
  - Flagella → motility
  - Spirochetes- spiral structures





BRIGHT FIELD (LIGHT MICROSCOPE)

- Light source → light goes via condenser → to stage where slide is present → light to lens system → to objective lens
- Light hits the specimen → light travels via objective and eyepiece lens system → resulting in a bright image
- Bacterial motility can be observed

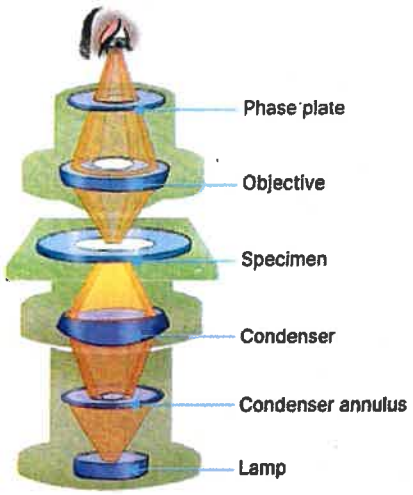
DARK FIELD

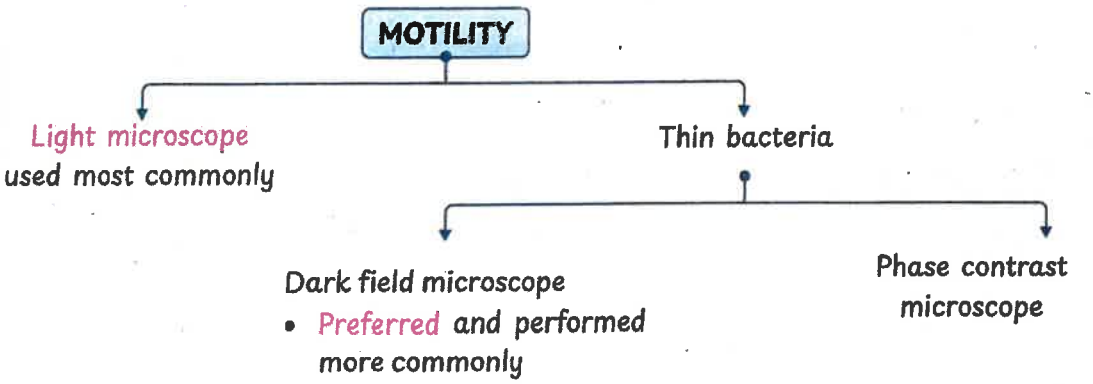
- Light source → light goes via condenser → to stage where slide is present → light to lens system → to objective lens
- A stop (central aperture) in the condenser blocks direct light from passing through the center
- This creates a hollow cone of light, which results in a dark background
- Used to visualize the motility of thin bacteria such as spirochetes

PHASE CONTRAST MICROSCOPE

00:16:57

- Similar to light microscope except for the following
  - Annular diaphragm (annular ring) in front of condenser
  - Annular phase plate
- Purpose of having different phases is → done when dealing with different refractive indices
- when light switched on → initially all the light waves are in the same phase → as it hits the specimen, having different refractive indices → considerable phase shift in light → light passes through the phase plate → intensity changed → shows different intensities of light
- Phase shift in light happens as specimen has different structures having different refractive indices
- Uses of phase contrast microscope
  - To see live organisms (unstained specimen) → as there is no need to add any dye to create contrast, contrast has already been created by the change in the phase of the light
  - To visualize the internal structure of the cell





FLUORESCENCE MICROSCOPE

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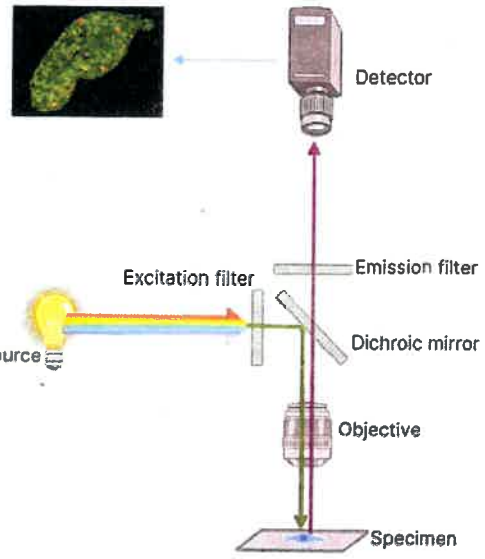


- Light used- mercury lamp → create ultraviolet light (not in visible range)
- Ultraviolet light passes through → excitation filter
- To decide which excitation filter to use → depends on the kind of dye used in specimen
- Dye → Fluorophores added to the specimen
- From excitation filter light strikes onto the dichroic mirror → light reflected towards the sample → dye in the sample changes the wavelength of light from shorter to a longer wavelength → longer wavelength of light emitted from the specimen passes through the emission filter → fluorescent picture seen
- Principle- shorter wavelength converted to longer wavelength of light

DYES/ FLUOROPHORES

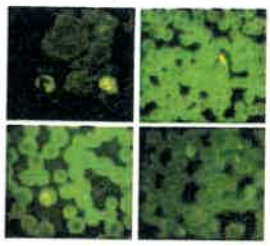
- Mycobacterium tuberculosis- Auramine, Rhodamine
  - Binds to mycolic acid of TB
- Malaria- Acridine orange
  - Binds to DNA of malaria
- Fungus- Calcofluor white
  - Binds to chitin of fungus present in outer part of the fungus

Important Information

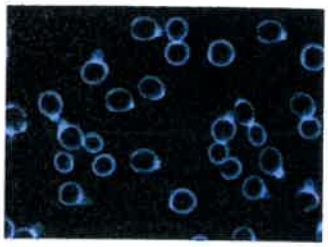
- Rhodanine is a stain for copper

MTB

- Rod shaped TB organisms seen
- Auramine rhodamine staining



- Ring forms of malaria
- schizont of malaria
- banana shaped gametocytes
- Acridine orange dye used



- Calcofluor white used
- Budding seen

- Fluorescent dye for flow cytometry - FITC: Fluoro Iso Thio Cyanate