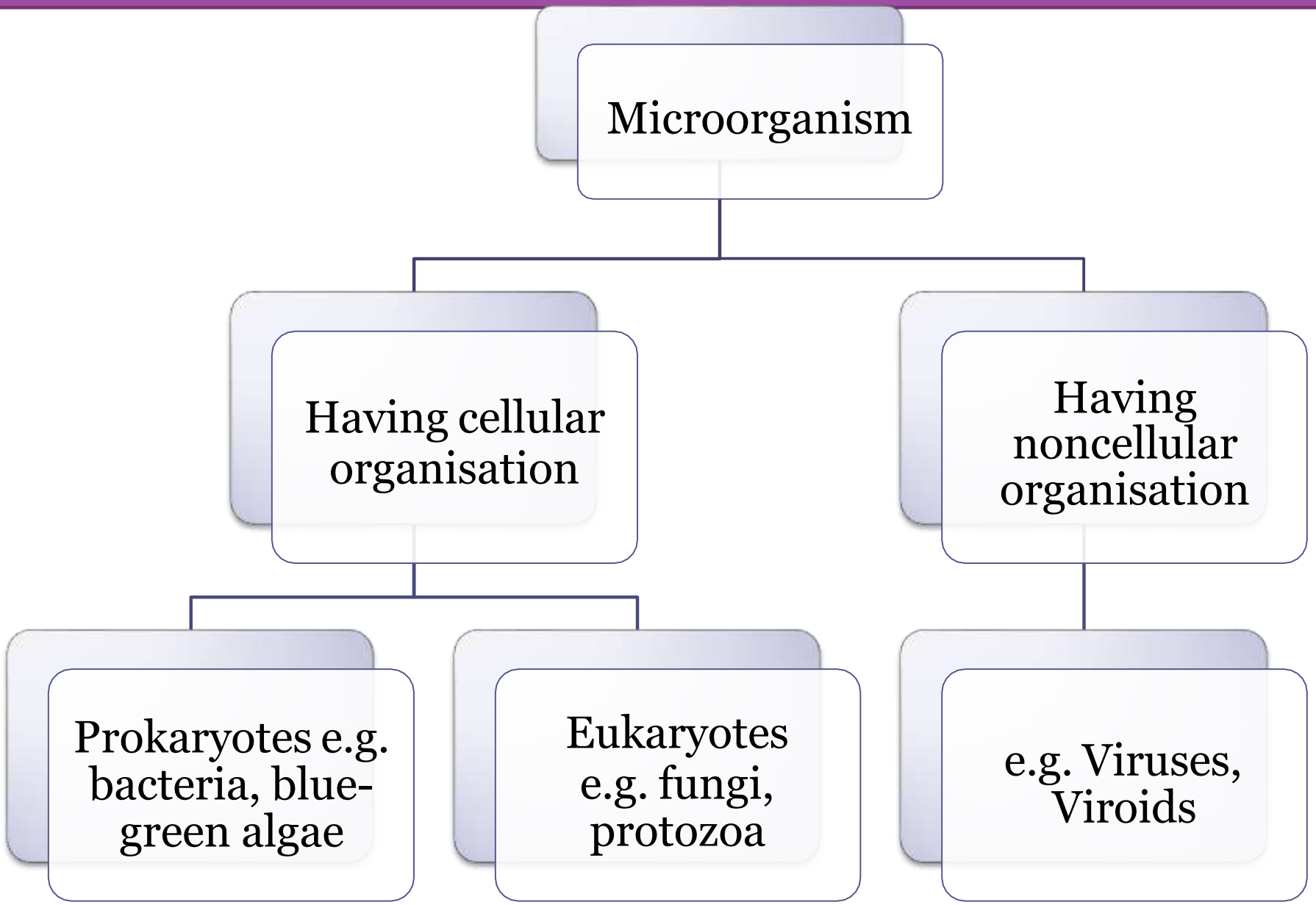
FUNDAMENTAL PRINCIPLES OF MICROBIOLOGY

Introduction

- Microbiology is the study of living organisms that are microscopic in size.
- Microbiology can be defined as the study of living organisms of microscopic size which include bacteria, fungi, algae, protozoa & viruses.
- Some microbes are beneficial & others are harmful to man.

Classification of microorganism



ISOLATION

- Isolation is defined as separating completely & obtaining in pure form of particular type of microorganisms, separating it from its habitat.

- Methods of isolation

✓Streak plate method
✓Pour plate method
✓Single cell isolation
✓Direct transfer technique

STREAK PLATE METHOD

- In this method small amount of sample transferred on a nutrient media in petridish.
- The sample is streaked by a Nichrome-wire loop in such a way that, streaking provides successive dilution & there by the isolated colonies.
- Streaking a solid culture medium in a petridish can be done by following methods

✓Square method

✓Four quadrant method

✓Zig-zag method

POUR PLATE METHOD

- In that technique serial dilutions of the given sample are necessary.
- A set of tubes each containing 9ml sterile distilled water is taken.
- Then 10ml of original sample is added to first tube containing distilled water. From this tube 1ml is transferred to second tube containing 9ml distilled water & so on.
- Finally 1ml of any suitable dilution is mixed with nutrient medium in petridish.
- The contents are mixed thoroughly by gentle rotation of petridish.
- Then, such plates incubate for specific time of period.

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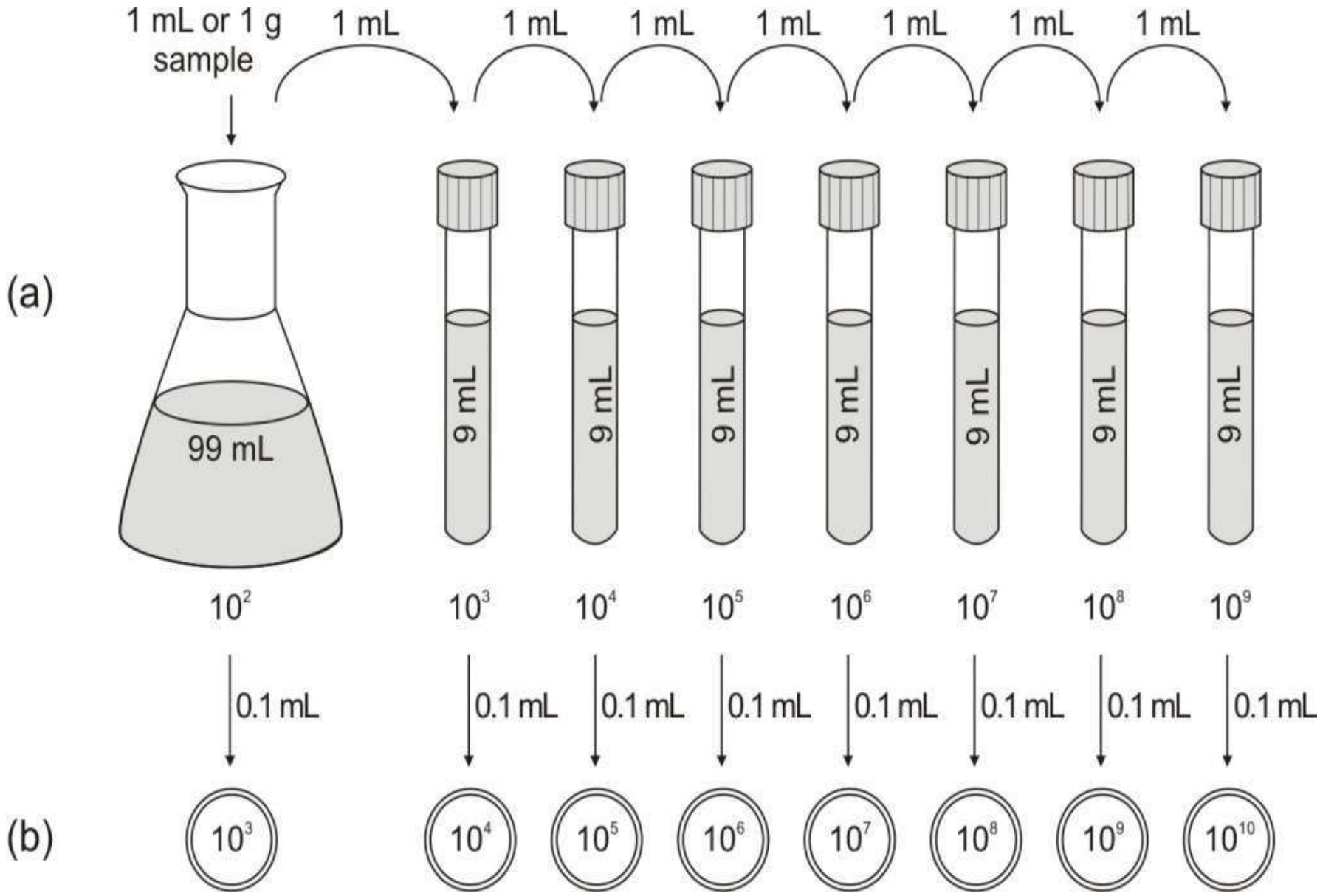
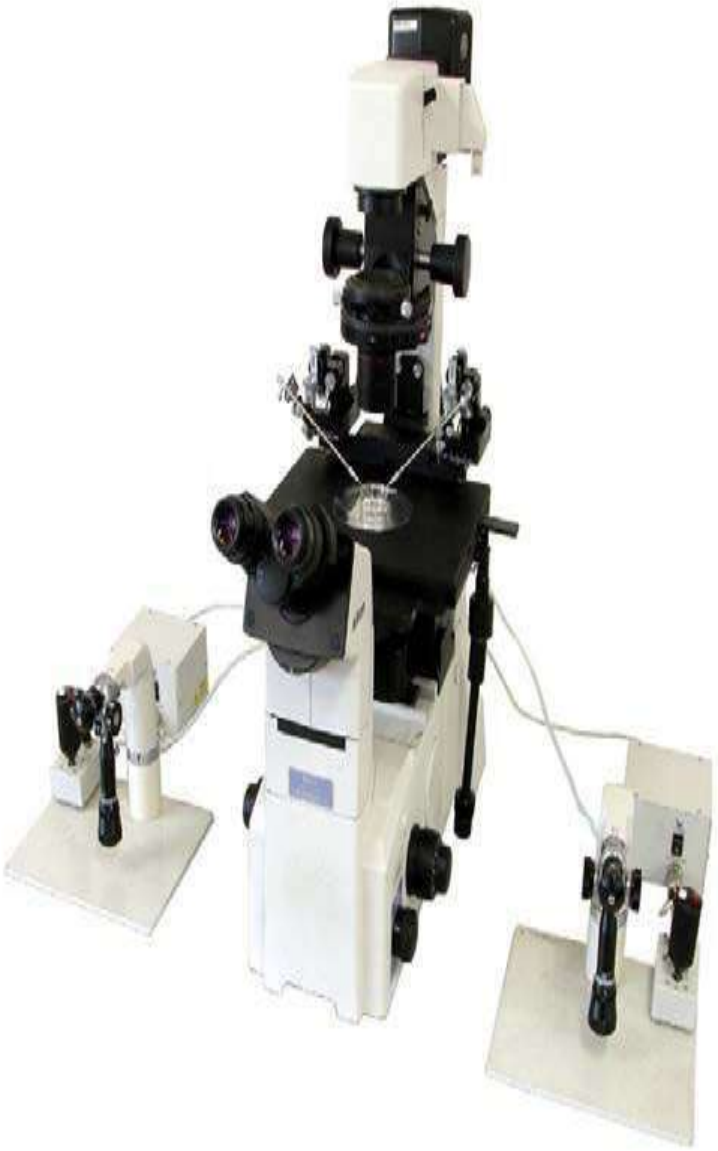

1 mL or 1 g sample
1 mL
1 mL
1 mL
1 mL
1 mL
1 mL
1 mL
(a)
99 mL
9 mL
9 mL
9 mL
9 mL
9 mL
9 mL
9 mL
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
0.1 mL
0.1 mL
0.1 mL
0.1 mL
0.1 mL
0.1 mL
0.1 mL
0.1 mL
(b)
10^3
10^4
10^5
10^6
10^7
10^8
10^9
10^{10}

Single cell isolation by micromanipulator

- In this method single cell can be pick out from the mixed culture.
- This can be done by micromanipulator combination with microscope.
- Then transferred to a suitable nutrient medium.



STAINING TECHNIQUES

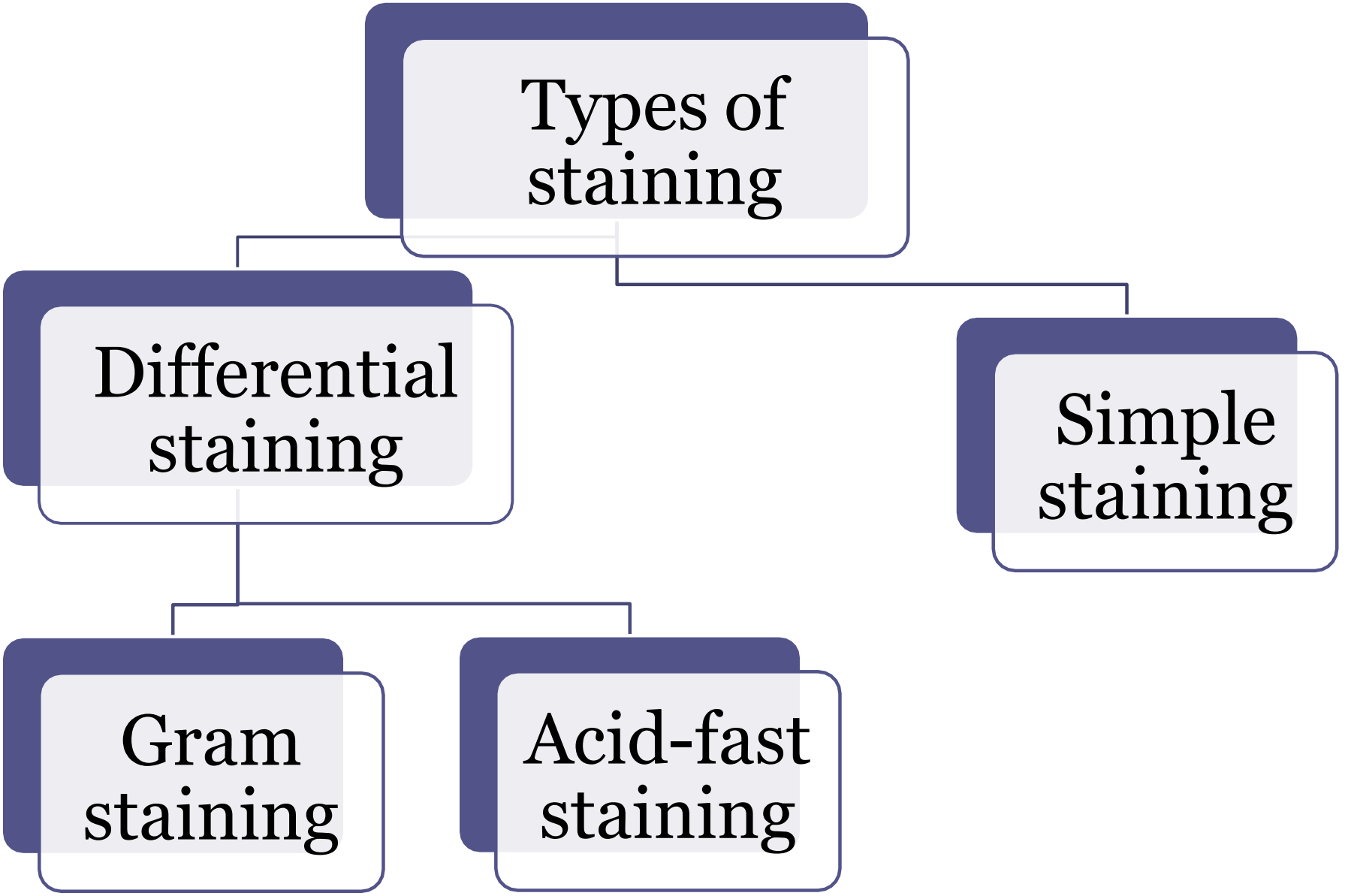
- Purpose of staining:

✓For greater visualization of cells.

✓For study of their structures

✓To differentiate the cells

- Stains : stains are the organic dyes used for staining the microorganisms.
- e.g. crystal violet, methylene blue etc.


Types of staining
Differential staining
Simple staining
Gram staining
Acid-fast staining

Gram Staining Procedure

Christian gram was firstly discovered the differential staining techniques, to differentiate types of bacteria.

✓ Gram-positive staining:

i. First take the bacteria & spread on a clean slide.
ii. This smear is dried by passing it over blue flame.
iii. Add crystal violet solu. & allow for 30-60 sec.
iv. Remove excess stain with water.
v. Then add iodine solu. & allow for 60 sec.
vi. Wash with water.
vii. Finally wash the slide with alcohol.
viii. Wash with water & observe under microscope.

Cont..

- Observation:

If stain appears a deep violet or purple black, then they are called as gram-positive bacteria.

e.g. staphylococci, pneumococci etc.

✓Gram-negative staining:

i. Staining procedure is similar to gram-positive.
ii. If bacteria do not retain crystal violet stain then it is counter stained by safranin for 10 sec.
iii. Washed with water & observe the smear under microscope.

- Observation : if pink or red colour produced then those are gram-negative bacteria. e.g. E.coli, S.typhi etc.

Ziehl Neelsons acid fast staining method

- Certain organisms are not easily stained by the usual dyes probably due to the presence of a water repellant outer layer or high lipid content , but when stained with acid fast stain they may retain the colour even washed with acid.

- *Mycobacterium tuberculosis & Mycobacterium leprae.*

- Ziehl & Neelsons discovered this method for separation of “Mycobacterium Group”.

Procedure…

- A smear is prepared.
- Then added Ziehl-Neelson carol fuchin stain ,for 10mint.
- Then heated & wash with tap water.
- Then add 20% H_2SO_4 for one mint & wash with water.
- Then add methylene blue for 30 sec.
- Wash the slide & dry & observe under oil immersion lens.

- OBSERVATION: cells appears pinkish red are acid fast cells/bacteria & those appears blue/green are nonacid fast bacteria.

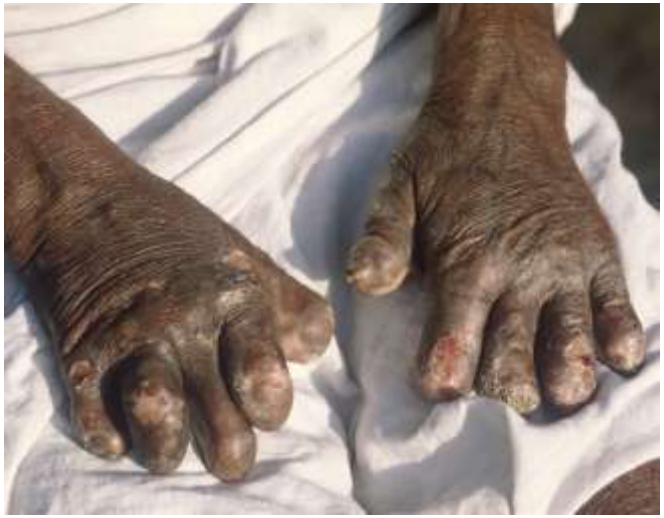
Diseases Produced by Bacteria

NAME OF BACTERIA	DISEASES PRODUCED
Staphylococci	Localised suppurative lesions
Streptococci	Rheumatic fever
E-coli	Urinary tract infection, Gastroenteritis
Clostridium tetani	Tetanus
Bcillus anthracis	Anthrax
Pneumoccoci	Pneumonia
Salmonella	Typhoid
Vibriocholera	Cholera
Mycoplsma pneumonae	Pneumonia
Mycobacterium leprae	Leprosy
Shigella	Dysentery
Haemophilus influenza	influenza

Cont..

TETANUS

ANTHRAX



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