

❖ IDENTIFICATION OF BACTERIA

❖ INTRODUCTION

Identification of unknown bacterial culture is one of the major responsibilities of microbiologist. Sample of blood, water, tissue, food, cosmetics are examine daily in laboratories. Bacteria are identified by staining technique and biochemical test.

❖ What is bacteria?

Bacteria are unicellular, free living, microscopic microorganisms capable of performing all essentials functions of life.

Fig.1)S.Aureus

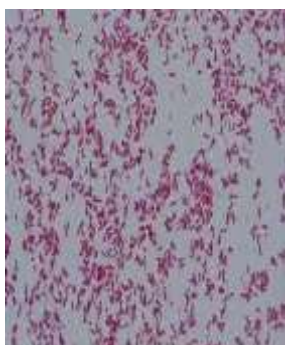
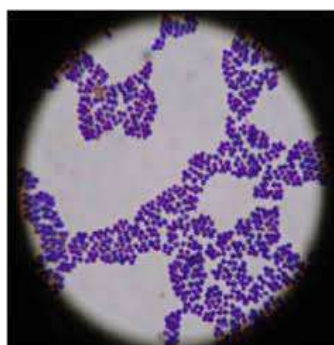


Fig.2)E.Coli



❖ MORPHOLOGICAL CLASSIFICATION OF BACTERIA

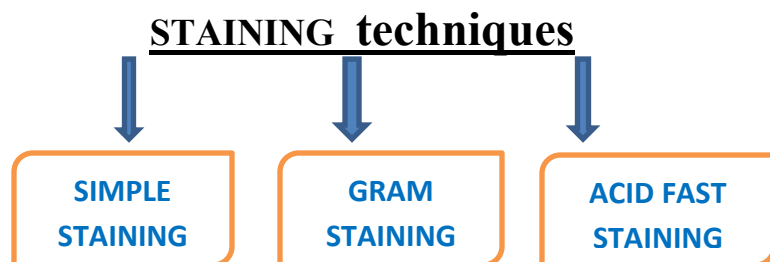
Bacteria are classified into five groups according to their shapes; Spherical (cocci), Rod (bacilli), Spiral (spirilla), Comma(vibrios) or corkscrew (spirochaetes), Actinomycetes, mycoplasma. They can exist as a single cells, in pairs, chain or clusters.

TABLE No- 1.1 Morphology of Bacteria

COCCI	Oval shape cells	Eg: Streptococcus lactis
BACILLI	Rod shape cells	Eg: Bacillus
SPIRILLA	Longer rigid rods with curves	Eg: Spirillum ruperm
VIBRIO	Comma shaped	Eg: Vibrio comma
SPIROCHETE	Slender and flexible	Eg: Treponema
ACTINOMYCETES	Branching filamentous	Eg: Streptomyces species
MYCOPLASMA	Cell wall deficient bacteria	Eg: Mycoplasma pneumonia

❖ STAINING REACTIONS

To study the size, shape, arrangement and properties and differentiate specific group of microorganisms, biological stains are used.



❖ SIMPLE STAINING

Simple staining involves directly staining bacterial cell with positively charged dye in order to see bacterial detail.



Fig.3) simple staining

❖ GRAM STAINING

Gram stain or Gram staining, also called Gram's method, is a method of staining used to classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

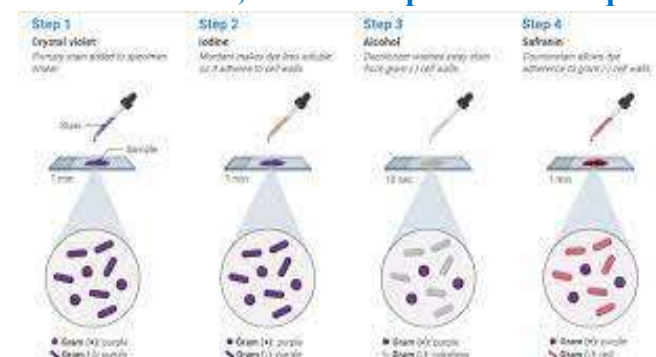


Fig.4) Procedure of gram staining

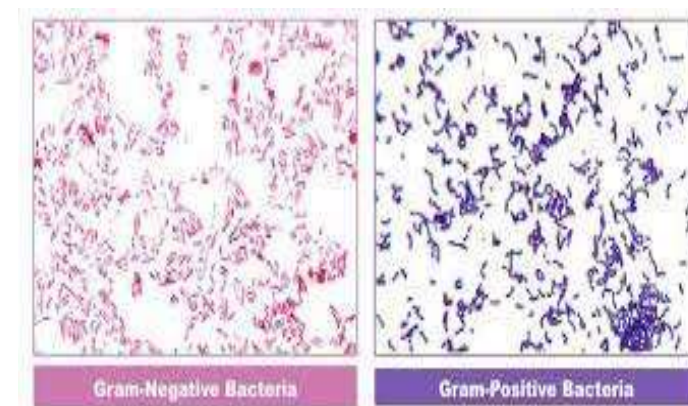


Fig.5) Gram staining

❖ ACID FAST STAINING

- It is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen.
- So this method is also called Ziehl-Neelsen staining techniques. Neelsen in 1883 used Ziehl's carbol-fuchsin and heat.
- Decolorized with an acid alcohol, and counter stained with methylene blue.
- Thus Ziehl-Neelsen staining techniques was developed.

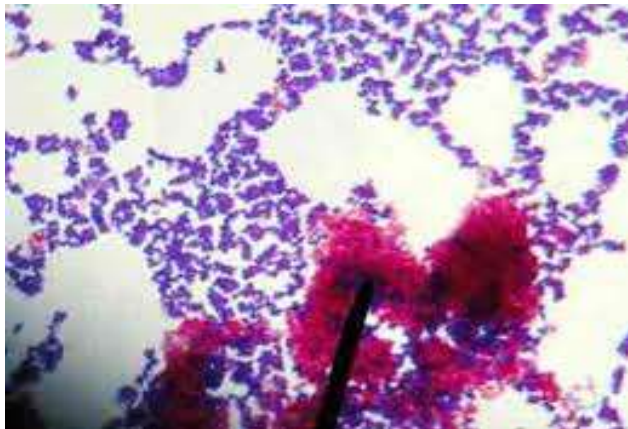


Fig6).Acid fast staining

❖ BIOCHEMICAL TEST-

IMVIC TEST-

- Indole test
- Methyl red test
- Vogous proskauer test
- Citrate test

TABLE NO.1.2 IMVIC TEST

Indole Test	Takesim media, inoculation neddle. Incubate it for 24hours at 37 ° C	If pink colour form it is Indole positive
MR-VP Test	Take MR-VP broth and inoculate bacterium in broth with help of inoculation neddle.	Intense pink colour it indicate positive
Citrate Test	Simmon citrate agar slant streak bacterium broth.	Colour change royal blue.

❖ IMVIC TEST FOR E.COLI -

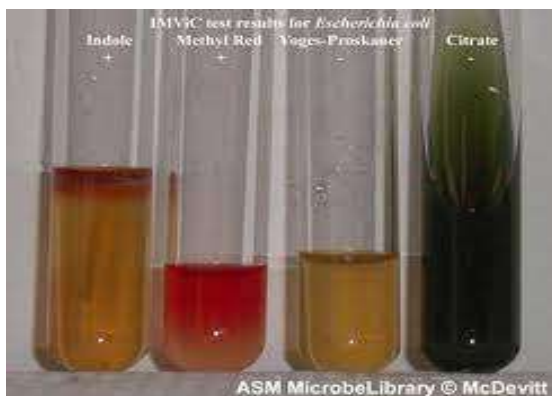


Fig no.7)IMVIC TEST

- **INDOLE TEST: Positive**
- **METHYL RED TEST:Positive**
- **VOGES-PROSKAUER TEST:Negative**
- **CITRATE-UTILIZATION TEST:Negative**

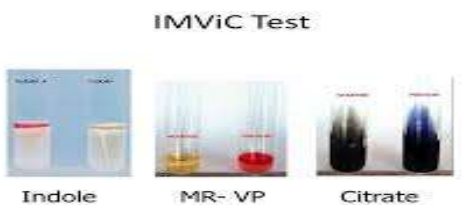


Fig no.8) IMVIC TEST

DIFFERENTIATION OF ENTERIC BACTERIA BY IMVIC TEST-

Table no1.3

GENUS	INDOLE	MR	VP	CITRATE
<i>Escherichia</i>	+	+	-	-
<i>Enterobacter</i>	-	-	+	+
<i>Klebsiella</i>	-	-	+	+
<i>Salmonella</i>	-	+	-	+
<i>Proteus</i>	+	+	-	+

REFERENCE-

- 1) Prof.C Kokare textbook of pharmaceutical microbiology, edition 2019 page no.89-98 by Nirali publication
- 2)Hugo and Russell pharmaceutical microbiology 7th edition page no.23-57 by BLACKWELL Publishing.

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