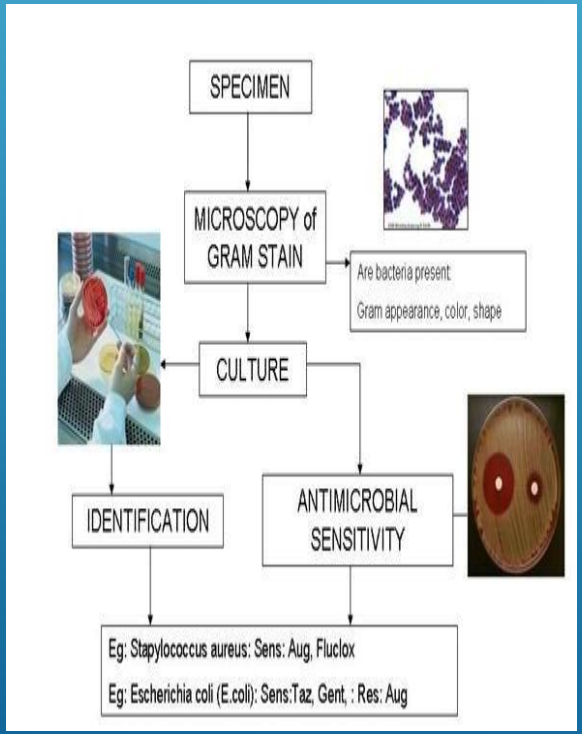
# CLASSIFICATION ,IDENTIFICATION OF BACTERIA AND THE LABORATORY DIAGNOSIS

| Taxonomy | Classification |
|---|---|
| Bacteria | Bacteria |
| Kingdom | Bacteria |
| Phylum | Firmicutes |
| Class | Bacilli |
| Order | Lactobacillales |
| Family | Streptococcaceae |
| Genus | *Streptococcus* |
| Species | *mutans* |




## *Bergey' s Manual of Determinative Bacteriology.*

***Taxonomy** is the science of classification of organisms*

***Bacterial taxonomy consists of three separate, but interrelated areas:***

- **Classification**
- **Nomenclature**
- **Identification**

*Classification is the arrangement of organisms into groups (taxa) on the basis of similarities or relationships.

*Nomenclature is the assignment of names to the taxonomic groups according to international rules

*Identification is the practical use of a classification scheme to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species.

# *Naming microorganisms*

- Binomial (scientific) nomenclature
- Gives each microbe 2 names
  - Genus - noun, always capitalized
  - species - adjective, lowercase
- Both italicized or underlined

*Staphylococcus aureus (S. aureus)*

*Bacillus subtilis (B. subtilis)*

*Escherichia coli (E. coli)*

# Taxonomic Rank

- Kingdom or Domain
- Division or Phylum
- Class
- Order
- Family
- Genus
- Species

| Formal rank | Example |
| --- | --- |
| Domain | Bacteria |
| Phylum | Gracilicutes |
| Class | Scotobacteria |
| Order | Eubacteriales |
| Family | Enterobacteriaceae |
| Genus | *Escherichia* |
| Species | *Coli* |

# Species

The basic and most important taxonomic group in bacterial systematics.

The boundaries of species are rather difficult to define precisely however; the boundaries of some genera are sharply defined. For example, the genus Bacillus and the genus Escherichia

The **successful identification** of microbiological agent depends on:

- proper aseptic **techniques**.
- Correctly obtaining the specimen.
- Correctly handling **the specimen**
- Quickly transporting the specimen to the lab.
- Once reaches the lab it is cultured and identified.

**After the microbe is identified, it is used in susceptibility tests to find out the effective control measure**

# Adansonian classification:

- In most systems of bacterial classification, the major groups are distinguished by fundamental characters such as cell shape, Gram-stain reaction and spore formation

- Genera and species are usually distinguished by properties such as fermentation reactions, nutritional requirements and pathogenicity.

# THE METHODS USE TO IDENTIFY BACTERIA FALL INTO THREE CATEGORIES

1. Phenotypic classification:
   - Morphology( macro and microscopic)
   - Microscopy  Gram staining characteristic
   - Growth requirement and metabolic behavior.

( biochemical test methods)

2. Immunological ( serological ) tests.
3. Genotypic- Molecular techniques.

# Steps in Diagnosis of Bacterial diseases

- Clinical Signs
- Laboratory examination

1- Microscopy

2- Culture techniques

3- Biochemical reactions

4- Serological identification:

5- Molecular biology techniques

6- Bacteriophage typing

# Microscopy

Microorganisms can be examined microscopically for:

a- Bacterial motility:

Hanging drop method:

A drop of bacterial suspension is placed between a cover slip and glass slid

b- Morphology and staining reactions of bacteria:

Simple stain: methylene blue stain

Gram stain: differentiation between Gm+ve and Gm–ve bacteria

. Primary stain (Crystal violet)

. Mordant (Grams Iodine mixture)

. Decolorization (ethyl alcohol)

. Secondary stain ( Saffranin)

Ziehl-Neelsen stain: staining acid fast bacilli

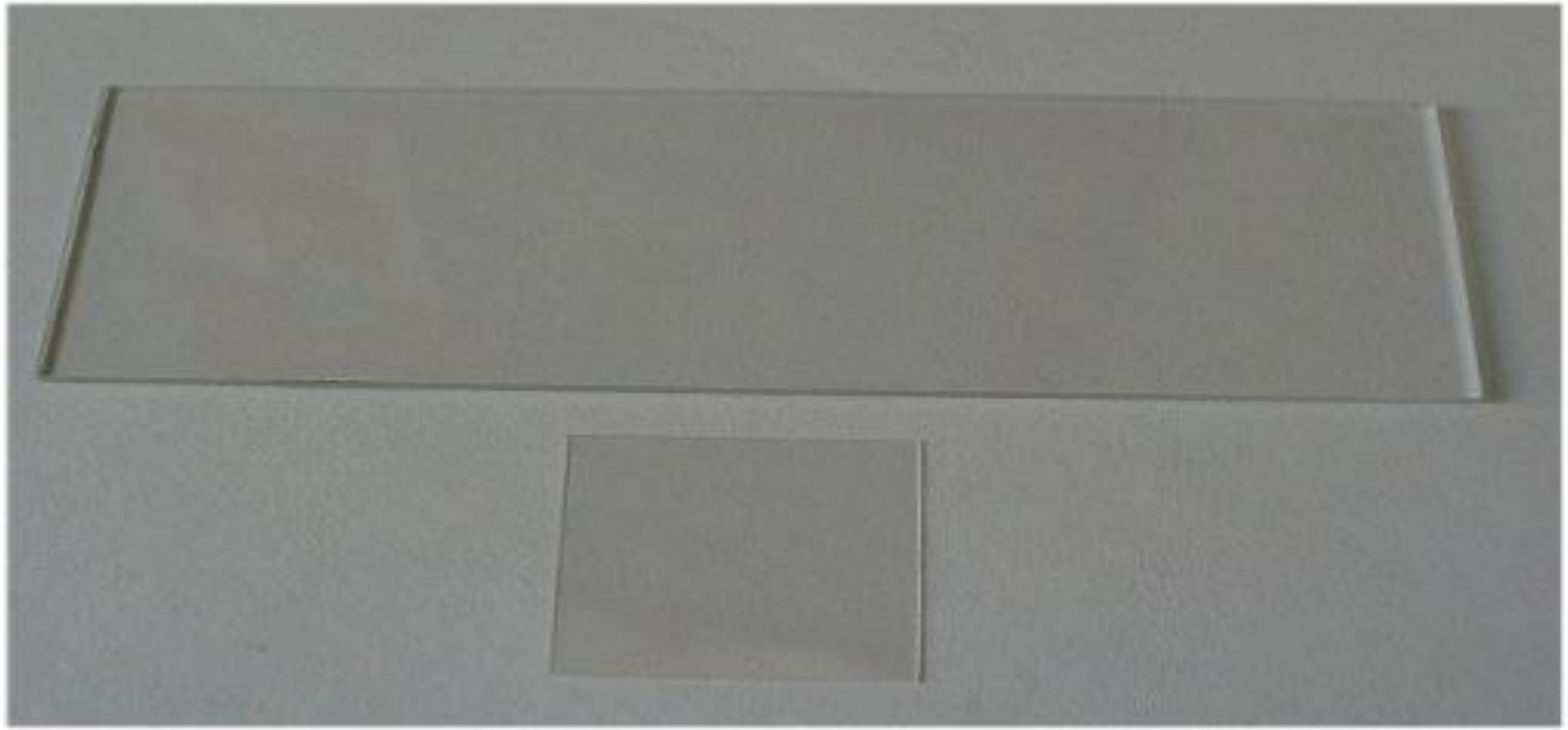
. Apply strong carbol fuchsin with heat

. Decolorization ($H_2SO_4$ 20% and ethyl alcohol

. Counter stain (methylene blue)

- The simple stain can be used as a quick and easy way to determine cell shape, size and arrangements of bacteria. True to its name, the simple stain is a very simple staining procedure involving single solution of stain. Any basic dye such as methylene blue, safranin, or crystal violet can be used to color the bacterial cells.

# Microscope glass slide and cover slip



# Culture for bacteria

- Sample is inoculated for culture and identification either in pre-enrichment or selective enrichment for broth culture. Incubated at suitable temperature for suitable time in proper environment
- Streaked on either selective, differential or both type of agar media for suitable time in proper environment
- Individual colonies are picked and grown as a pure culture.
- Tentative ID made based on colony shape and staining.
- Definitive ID requires biochemical, serological, and various tests.

# Culture Techniques

* Culture media are used for:
  - Isolation and identification of pathogenic organisms
  - Antimicrobial sensitivity tests
* Types of culture media:
  a- Liquid media:
    - Nutrient broth: meat extract and peptone
    - Peptone water for preparation sugar media
    - Growth of bacteria detected by turbidity
  b- Solid media:
    - Colonial appearance
    - Hemolytic activity
    - Pigment production

## Hemolysis on blood agar:

- **Complete (beta) hemolysis**:
  - Staphylococcus aureus and Streptococcus pyogenes.

- **Partial (alpha) hemolysis**:
  - Streptococcus viridans and pneumococci.

- **No (gamma) hemolysis:**
  - Enterococci.

# Types of solid media

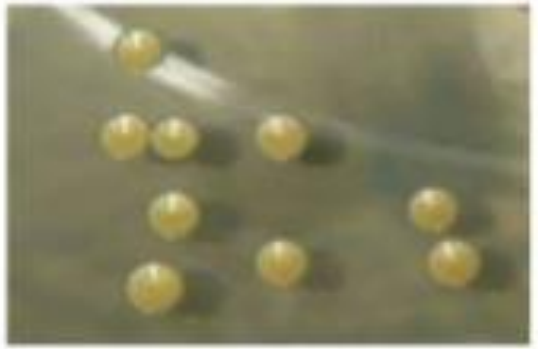
1- Simple media:
Nutrient agar
2- Enriched media: media of high nutritive value
. Blood agar
. Chocolate agar

3- Selective media: allow needed bacteria to grow
. Lowenstein–Jensen medium
. MacConkey's agar
. Mannitol Salt Agar
4- Indicator media: to different. between lact. and non lact. ferment
. MacConkey's medium
. Eosine Methylene blue Agar
5- Anaerobic media: for anaerobic cultivation
. Deep agar, Robertson's Cooked Meat Medium

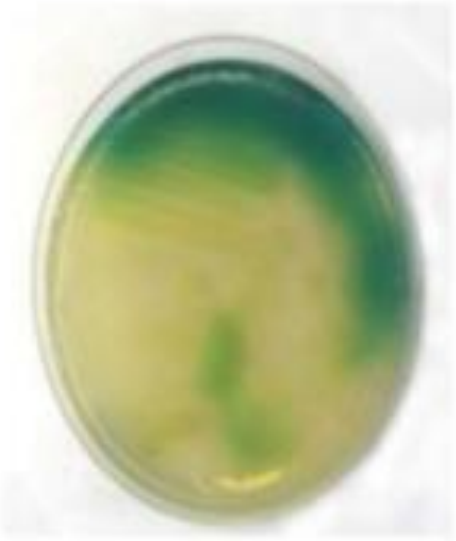
# Colonial appearance on culture media

* Colony morphology:
  . Shape . Size . Edge of colony . Color
* Growth pattern in broth:
  . Uniform turbidity
  . Sediment or surface pellicle
* Pigment production:
  . Endopigment production (*Staph. aureus*)
  . Exopigment production (*Ps. aeruginosa*)
* Haemolysis on blood agar:
  . Complete haemolysis (*Strept. pyogenes*)
  . Partial haemolysis (*Strept. viridans*)
* Growth on MacConkey's medium:
  . Rose pink colonies (Lactose fermenters)
  . Pale yellow colonies (Non lactose fermenters)

- **Pigment production:**
  - **Endopigment** (restricted to the colonies):
    - Golden yellow with *Staphylococcus aureus*.
    - White with *Staph. epidermidis.*

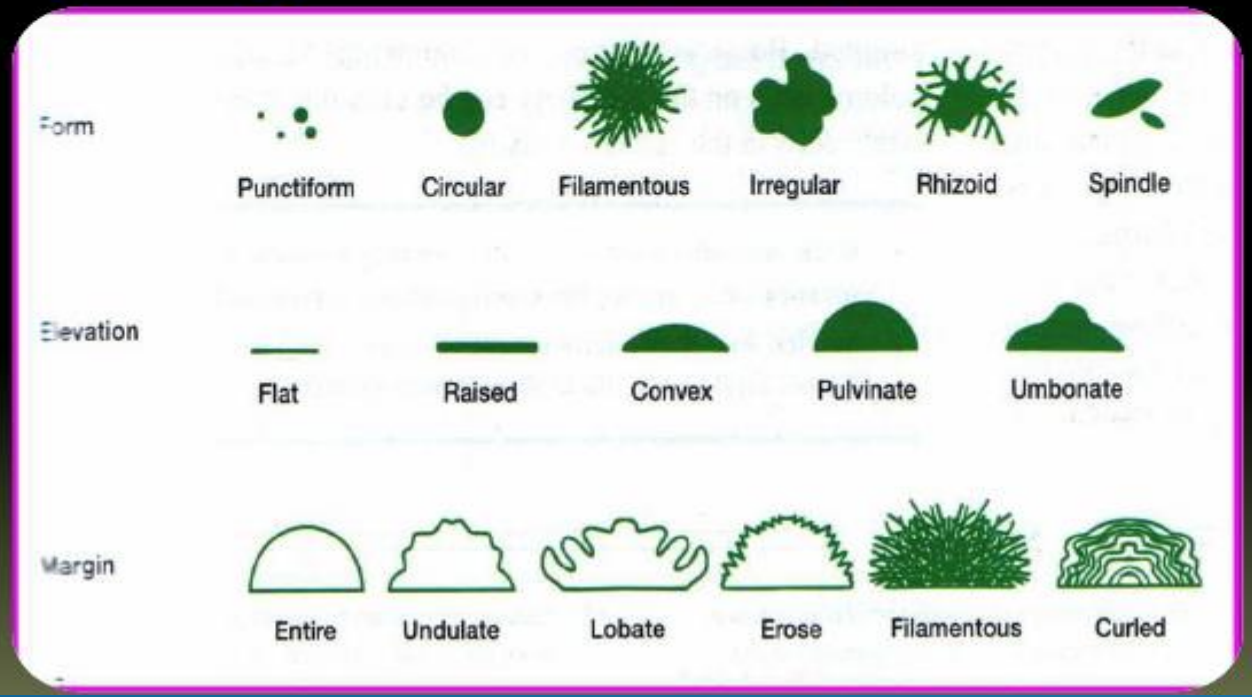


  - **Exopigment** (the color diffuses in the surrounding medium):
    - Green exopigment with *Pseudomonas aeruginosa*.

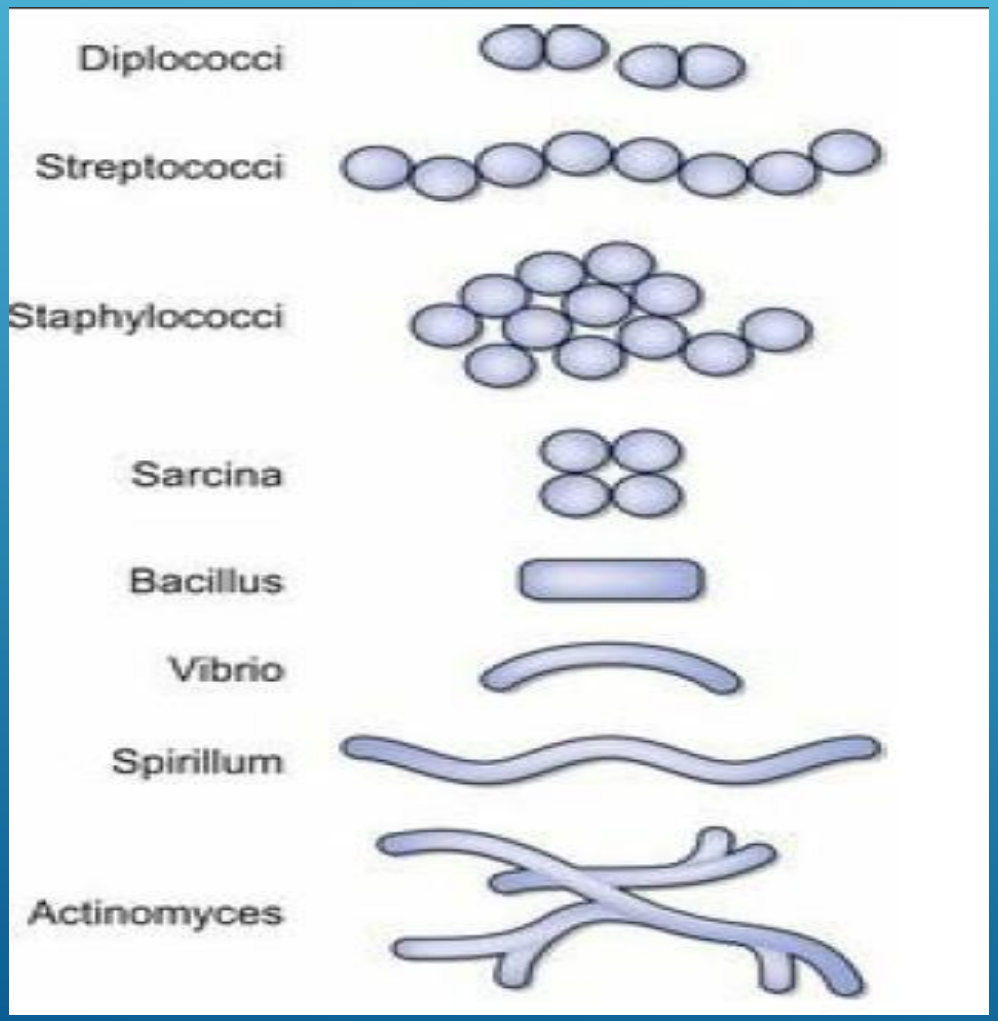


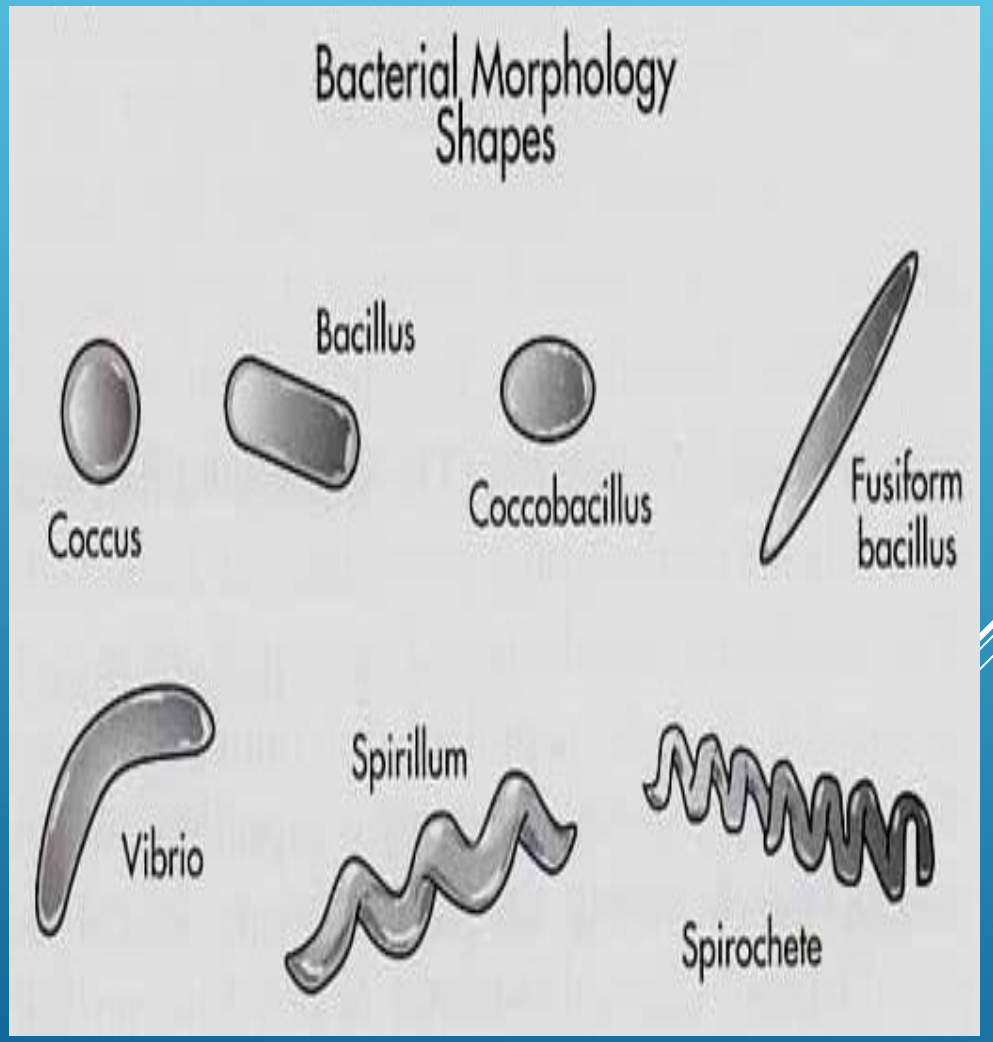
Colony characteristics : with the naked eye e.g. texture, shape, pigment, growth pattern.

- **Colony form**: pinpoint, circular, filamentous, irregular
- **Colony elevation:** flat, raised, convex
- **Colony margin:** smooth, irregular



# COLONY CHARACTERISTICS



# *Morphology:*

- Cocci
- Bacilli
- Curved or spiral
- Filamentous

* *Some correlation between morphology and disease e.g.*

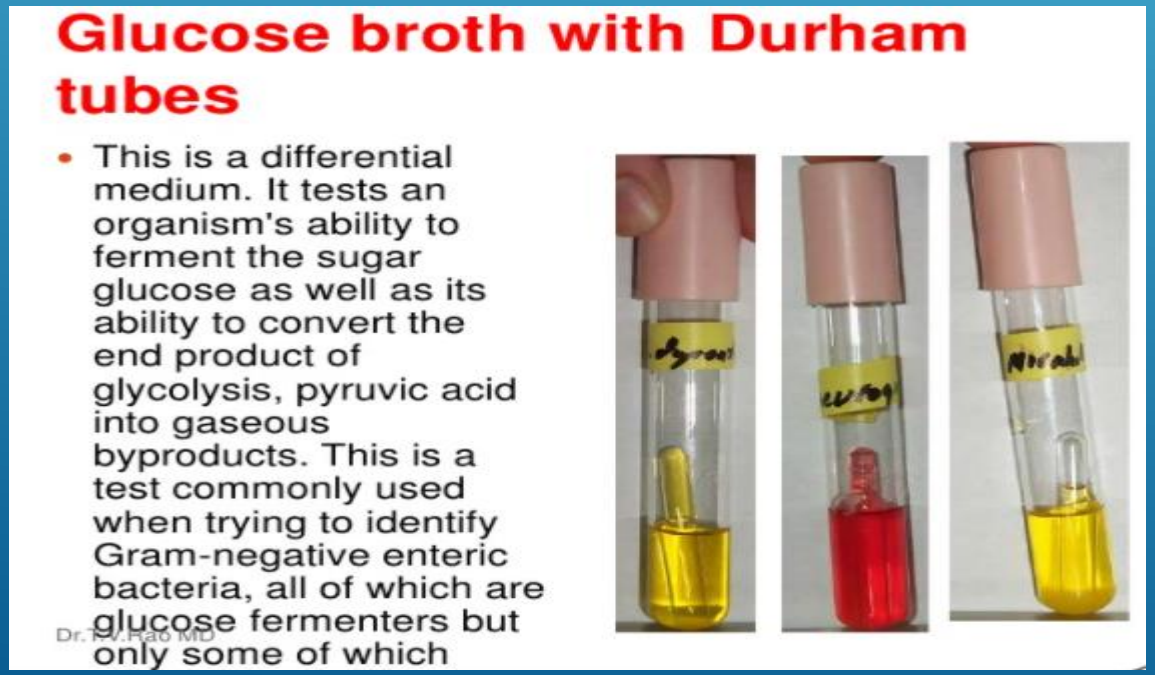
- ***Spiral bacteria*---Treponemes, Borrelias, Leptospiras tend to cause systemic diseases**
- ***Pathogenic Filamentous bacteria*--- Actinomyces, Nocardia, Mycobacteria tend to cause chronic diseases**
- ***Gram positive bacteria*--- Staphylococcus, Streptococci more likely to cause skin infections**

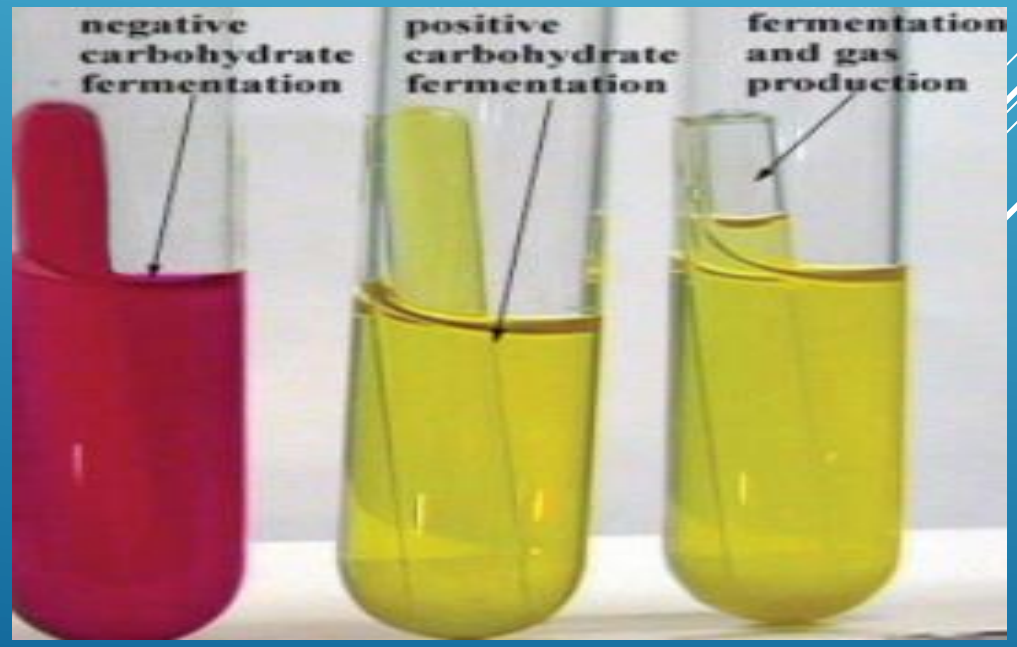
# BIOCHEMICAL REACTIONS

(Use of substrates and sugars to identify pathogens)

**Sugar fermentation:

- Organisms ferment sugar with production of acid only.
- Organisms ferment sugar with production of acid and gas.
- Organisms do not ferment sugar.

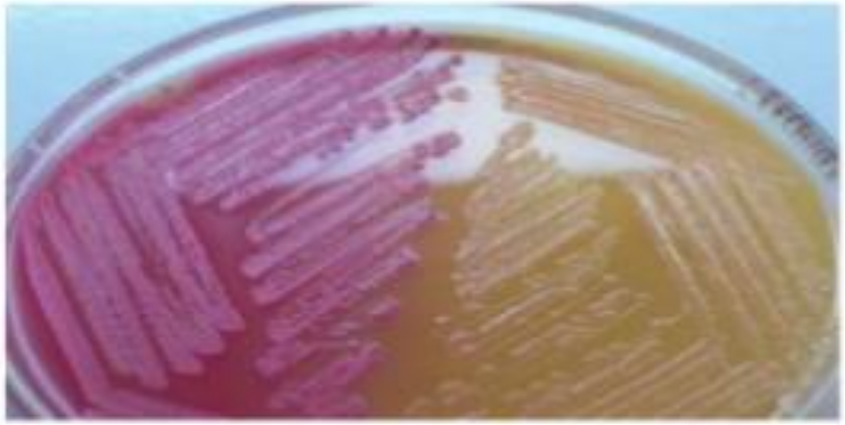


# Effect on lactose of MacConkey's agar:

- **Lactose fermenters**:
  - Appear as rose pink colonies.
  - Example: *E. coli* & *klebsiella*.

- **Non Lactose fermenters:**
  - Appear as pale colonies.
  - Example: *salmonella* & *shigella*.



** PRODUCTION OF INDOLE :

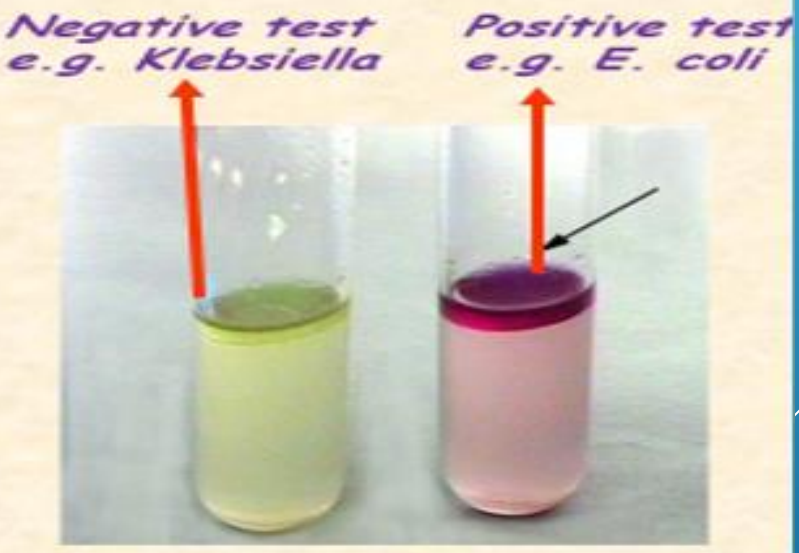
Depends on production of indole from amino acid tryptophan .

Indole is detected by addition of Kovac's reagent.

Appearance of red ring on surface.

## IMViC: Indole test

- **Result:**
  - A bright pink color in the top layer indicates the presence of indole
  - The absence of color means that indole was not produced i.e. indole is negative
- **Special Features:**
  - Used in the differentiation of genera and species. e.g. *E. coli* (+) from *Klebsiella* (-).

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# ** *HYDROGEN SULFIDE (H2S) PRODUCTION TEST*

determines whether the microbe reduces **sulfur-containing *compounds*** to **sulfides** during the process of metabolism.

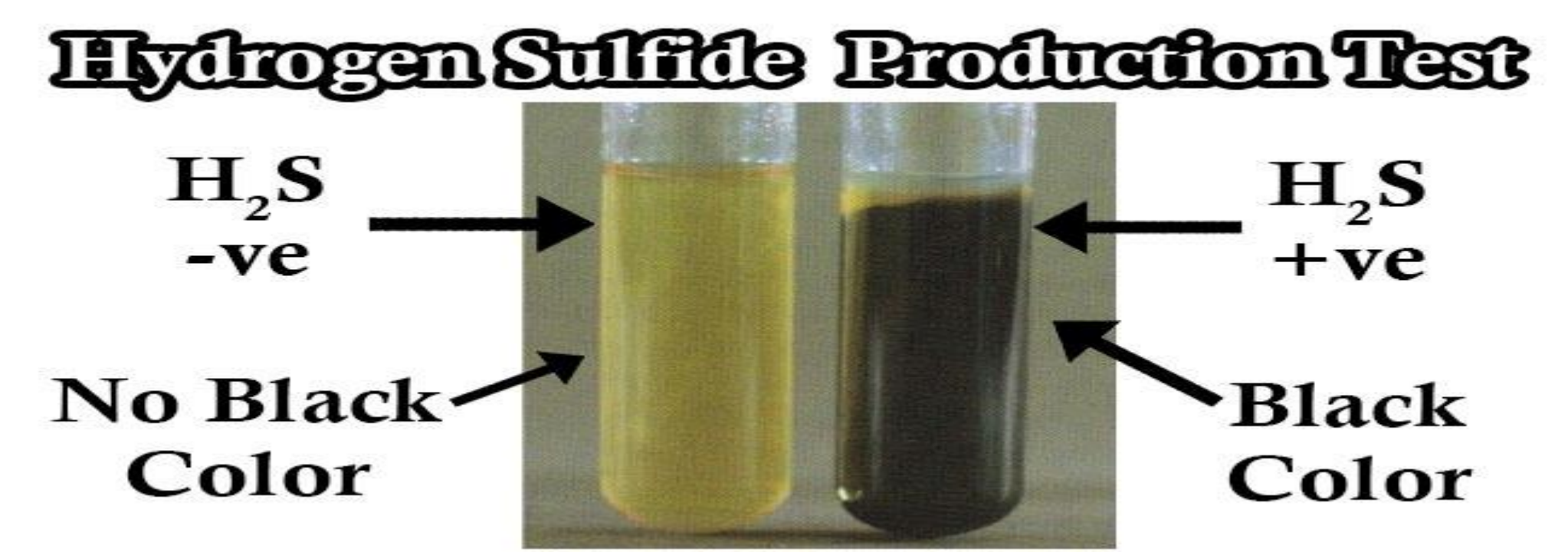
Several media containing ***iron compounds*** allow detection of **hydrogen sulfide** production.

One medium used is **Sulfide-Indole-Motility (SIM) medium.**

A second medium is **triple sugar iron agar (TSIA)**

The test aids in the identification and differentiation of members of Enterobacteriaceae (enterics) from other Gram- bacilli. It is especially helpful in identifying **Salmonella, Francisella, and Proteus species.**

# RESULT INTERPRETATION OF HYDROGEN SULFIDE (H2S) PRODUCTION TEST




**Positive result:** blackening on the medium
**Negative result:** no blackening on the medium

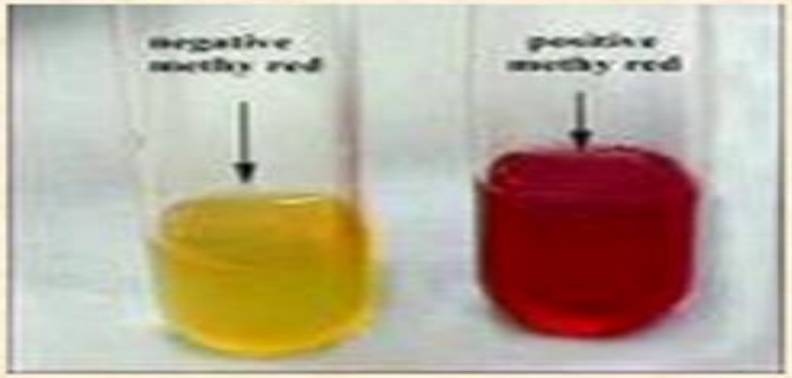
# Biochemical Reaction (cont.)

- Methyl red reaction (MR):
  Fermentation of glucose with production of huge amount of acid
  Lowering pH is detected by methyl red indicator

- Voges proskaur's reaction (VP):
  Production of acetyl methyl carbinol from glucose fermentation
  Acetyl methyl carbinol is detected by addition KOH
  Color of medium turns pink (positive)

## IMViC test: MR/VP test

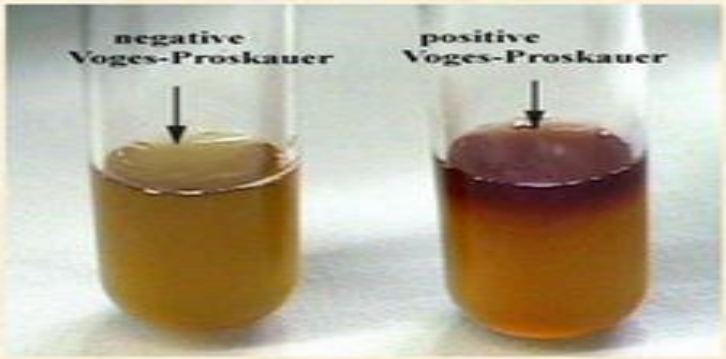
❖ *Results*




*Methyl Red test*

✓*Red: Positive MR (E. coli)*

✓*Yellow or orange: Negative MR (Klebsiella)*




*Voges-Proskauer test*

✓*Pink: Positive VP (Klebsiella)*

✓*No pink: Negative VP (E. coli)*

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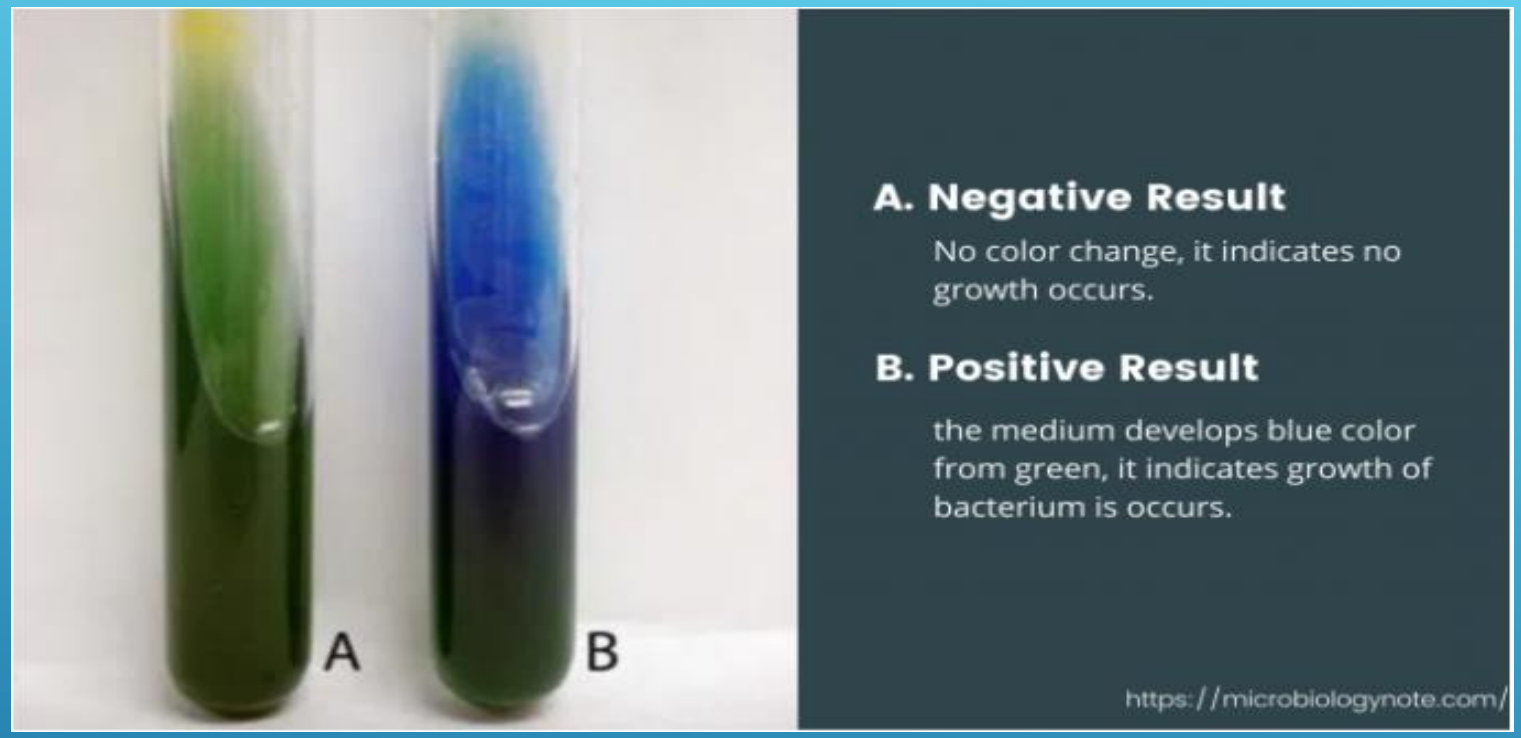
# Citrate utilization test




- It is an important test that allows the species-level identification of the members of the Enterobacteriaceae family.

**Objective**

•to detect the ability of organisms to produce citrase enzyme.

**Principle of citrate utilization test:**

The basic principle of this test is to detect the ability of an organism which can utilize citrate as a sole source of carbon for their metabolism with resulting alkalinity. The ***citrase enzyme*** hydrolyses the citrate to form oxaloacetic acid and acetic acid.

medium used is : Simmons citrate agar.

Oxidase test:

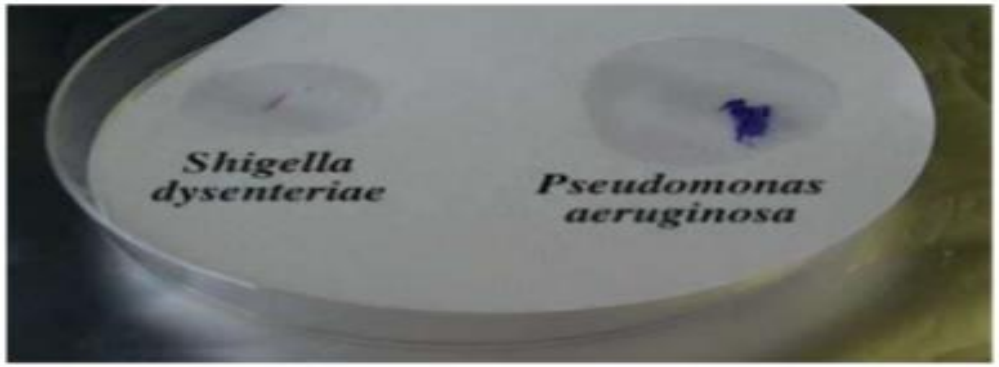
Some bacteria produce Oxidase enzyme

Detection by adding few drops of colorless Oxidase reagent

Colonies turn deep purple in color (positive)

## Oxidase Test

- All Enterobacteriaceae are **oxidase-negative.**
- This test is used to differentiate enterobacteriaceae from *Pseudomonas* which is **oxidase positive.**



- Catalase test:

Some bacteria produce catalase enzyme

Addition of $H_2O_2$ lead to production of gas bubbles ($O_2$ production)

- **Catalase test:**

- Is used to differentiate between staphylococci(catalase +ve) and streptococci(catalase –ve).

- Principle:

$$2\,H_2O_2 \xrightarrow{\text{Catalase enzyme}} 2\,H_2O + O_2$$

- Procedure
  - Smear a colony of the organism to a slide
  - Drop $H_2O_2$ onto smear
  - Observe

- Coagulase test:

  Some bacteria produce coagulase enzyme

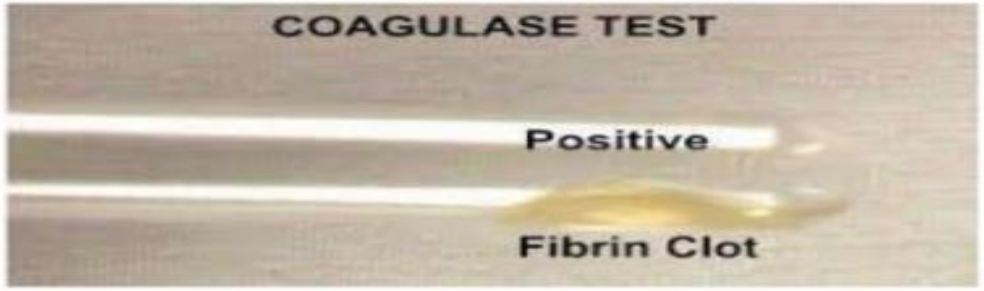
  Coagulase enzyme converts fibrinogen to fibrin (plasma clot)

  Detected by slide or test tube method

## Coagulase test

is used to differentiate *Staphylococcus aureus* from coagulase-negative staphylococci.

fibrinogen —(coagulase)→ fibrin (clot formation)



- Urease test:

Some bacteria produce urease enzyme

Urease enzyme hydrolyze urea with production of $NH_3$

Alkalinity of media and change color of indicator from yellow to pink

# Urease test

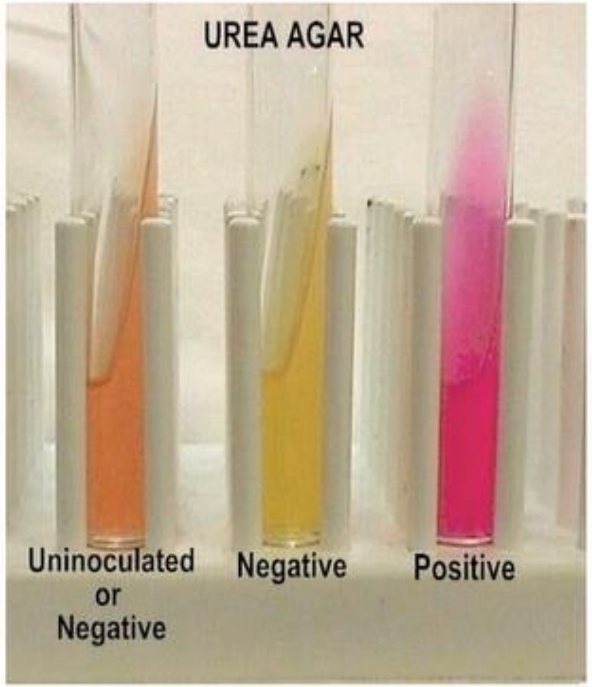
**Exercise-** inoculate

- E.coli, Proteus vulgais
- unknown

- Incubate 48 h at 37 $^0$c

**Interpretation:**

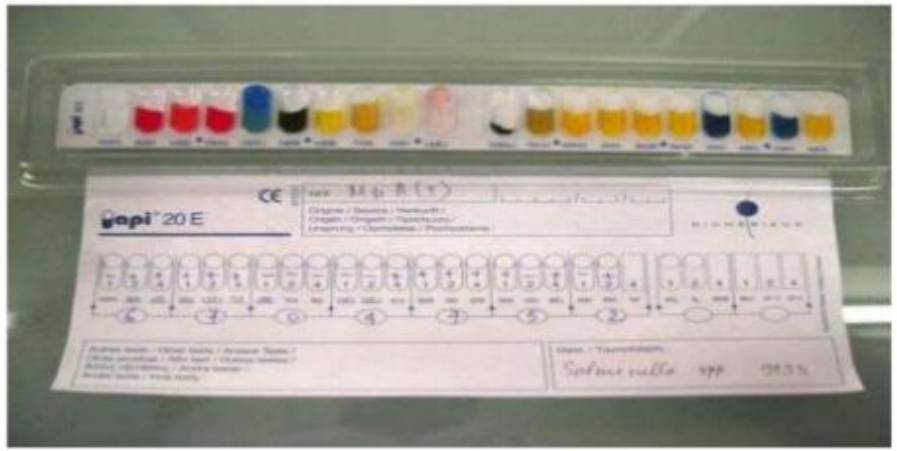
- The release of ammonia by the breakdown of urea results in the an alkaline pH of the medium which will turn the medium pink.
- Thus pink color indicates production of urease by the organisms
- Absence of pink color indicates a negative test for urease.




**Urease test Positive:** Red-pink color ( *Proteus vulgaris)*

**Urease test Negative:** No pink color ( *E. coli*)

## Analytical profile index (API):




# API System

- The **analytical profile index** or **API** is a classification of bacteria based on experiments, allowing fast identification. This system is developed for quick identification of clinically relevant bacteria. Because of this, only known bacteria can be identified.

# API 20E


- Aim
- Principle
- API test strips consists of microtubes (cupules) containing dehydrated substrates to detect the enzymatic activity
  fermentation of sugars
- During incubation, metabolism produces colour changes.
- When the carbohydrates are fermented, the pH within the cupule changes and is shown by an indicator.

# Automated bacterial identification systems:

- **Principle:**

- Examples: **Vitek system**
- These systems identify the organism and its antibiotic sensitivity by detecting color changes or turbidity in special plastic cards inoculated with the organism.

- Such cards are composed of tiny wells that contain substrates for detection of biochemical reactions and antibiotic sensitivity.

- Once the card has been inoculated and placed in the instrument, it will automatically perform all readings.

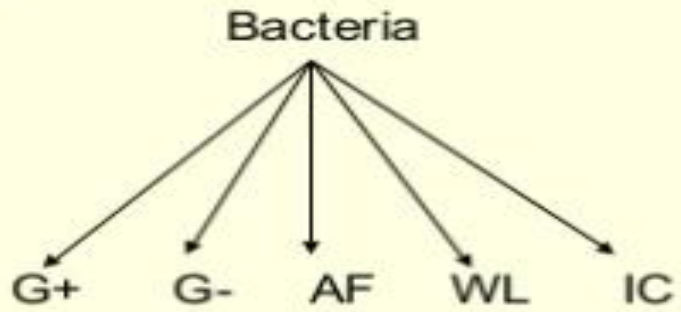
- Results are available within 4-6 hours.

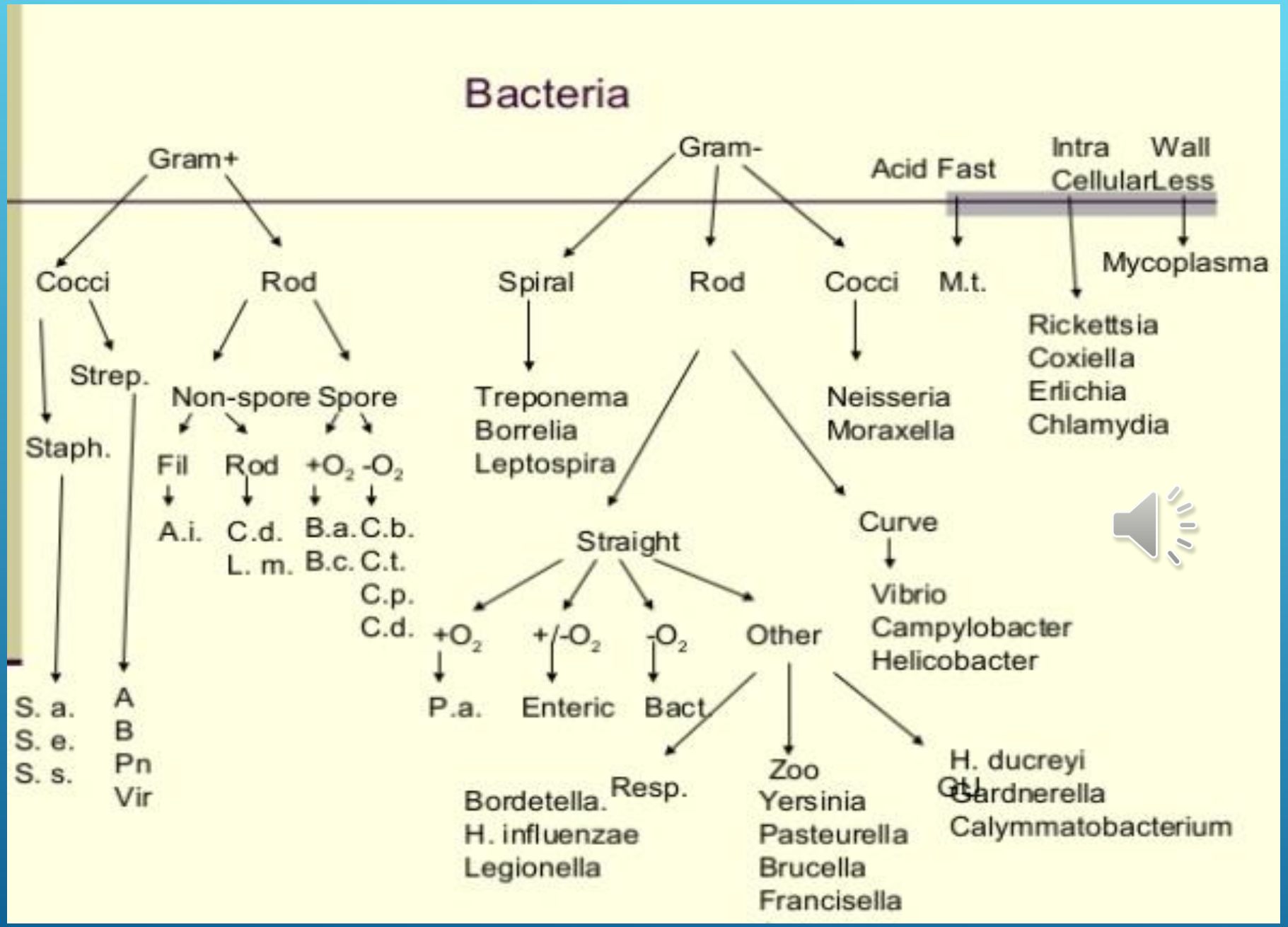
Vitek system

Vitek card

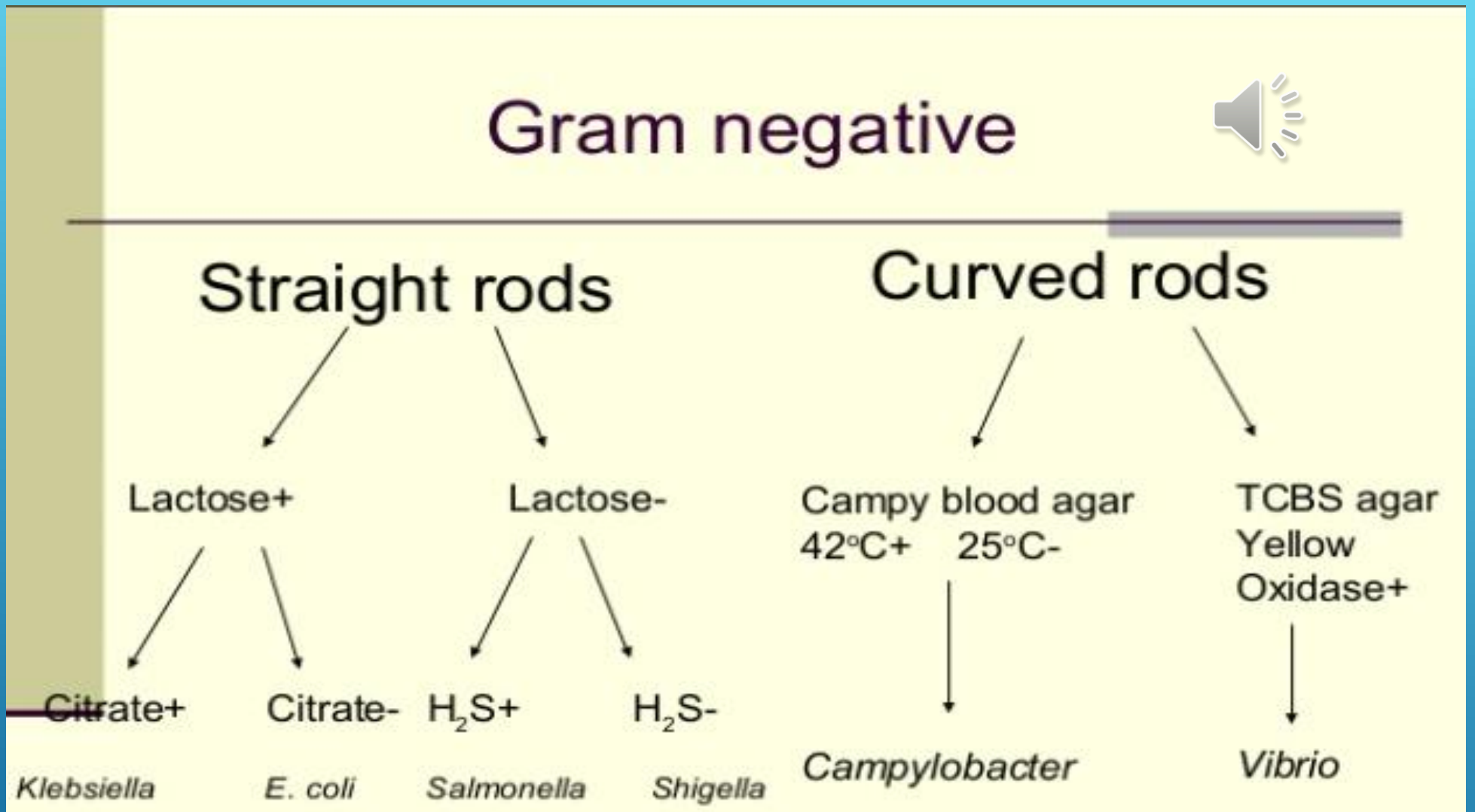
# Bacteria are of many types

- With Cell Wall
  - Gram +
    - *Staphylococcus*, *Streptococcus*, *Clostridium*, *Bacillus*
  - Gram -
    - Enteric, respiratory and others
  - Acid-fast
    - *Mycobacterium*
  - Wall-less
    - *Mycoplasma*
- Unusual
  - Obligate intracellular
    - *Rickettsia*, *Chlamydia*




Bacteria
Gram+
Gram-
Acid Fast
Intra Cellular
Wall Less
Cocci
Rod
Spiral
Rod
Cocci
M.t.
Mycoplasma
Rickettsia
Coxiella
Erlichia
Chlamydia
Strep.
Staph.
Non-spore
Spore
Treponema
Borrelia
Leptospira
Neisseria
Moraxella
Fil
Rod
+O2
-O2
A.i.
C.d.
L. m.
B.a.
B.c.
C.b.
C.t.
C.p.
C.d.
Straight
Curve
Vibrio
Campylobacter
Helicobacter
+O2
+/-O2
-O2
Other
P.a.
Enteric
Bact
S. a.
S. e.
S. s.
A
B
Pn
Vir
Resp.
Bordetella.
H. influenzae
Legionella
Zoo
Yersinia
Pasteurella
Brucella
Francisella
GU
H. ducreyi
Gardnerella
Calymmatobacterium

# Gram negative



## Animal inoculation

- The use of laboratory animals (mice, guinea pigs, rabbits) is now limited due to the advancement in medical microbiological techniques.

Guinea pig

Mouse

## But they could be used :

1. For growing the organisms that do not grow on culture such as lepra bacilli.

2. To determine the virulence factor of an organism. For example if injection of diphtheria in a guinea pig caused its death, this means that the organism is toxigenic.

# Evaluation of vaccines and antibiotics

# Immunological Methods

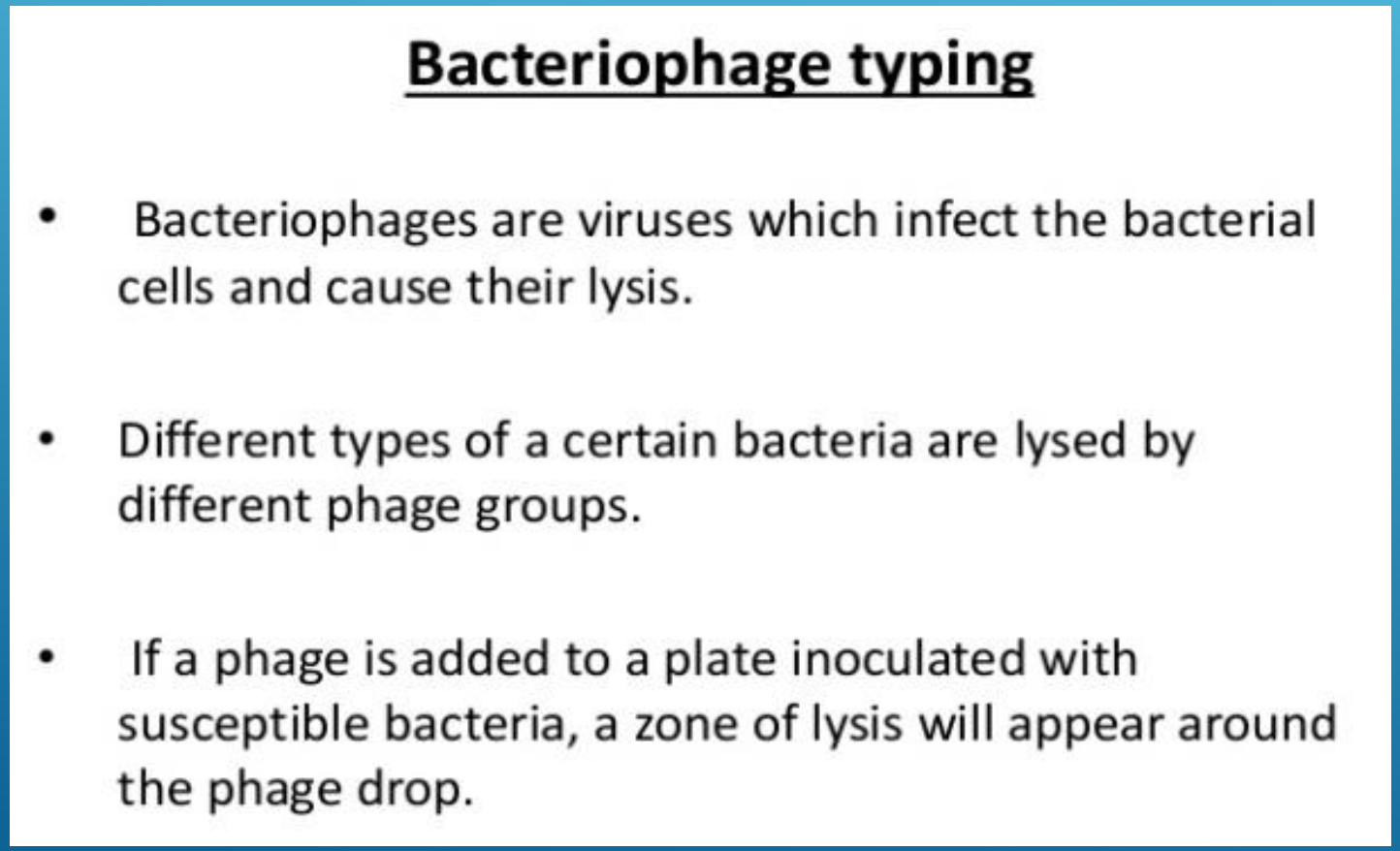
- Immunological methods involve the interaction of a microbial antigen with an antibody (produced by the host immune system).
- Testing for microbial antigen or the production of antibodies is often easier than test for the microbe itself.

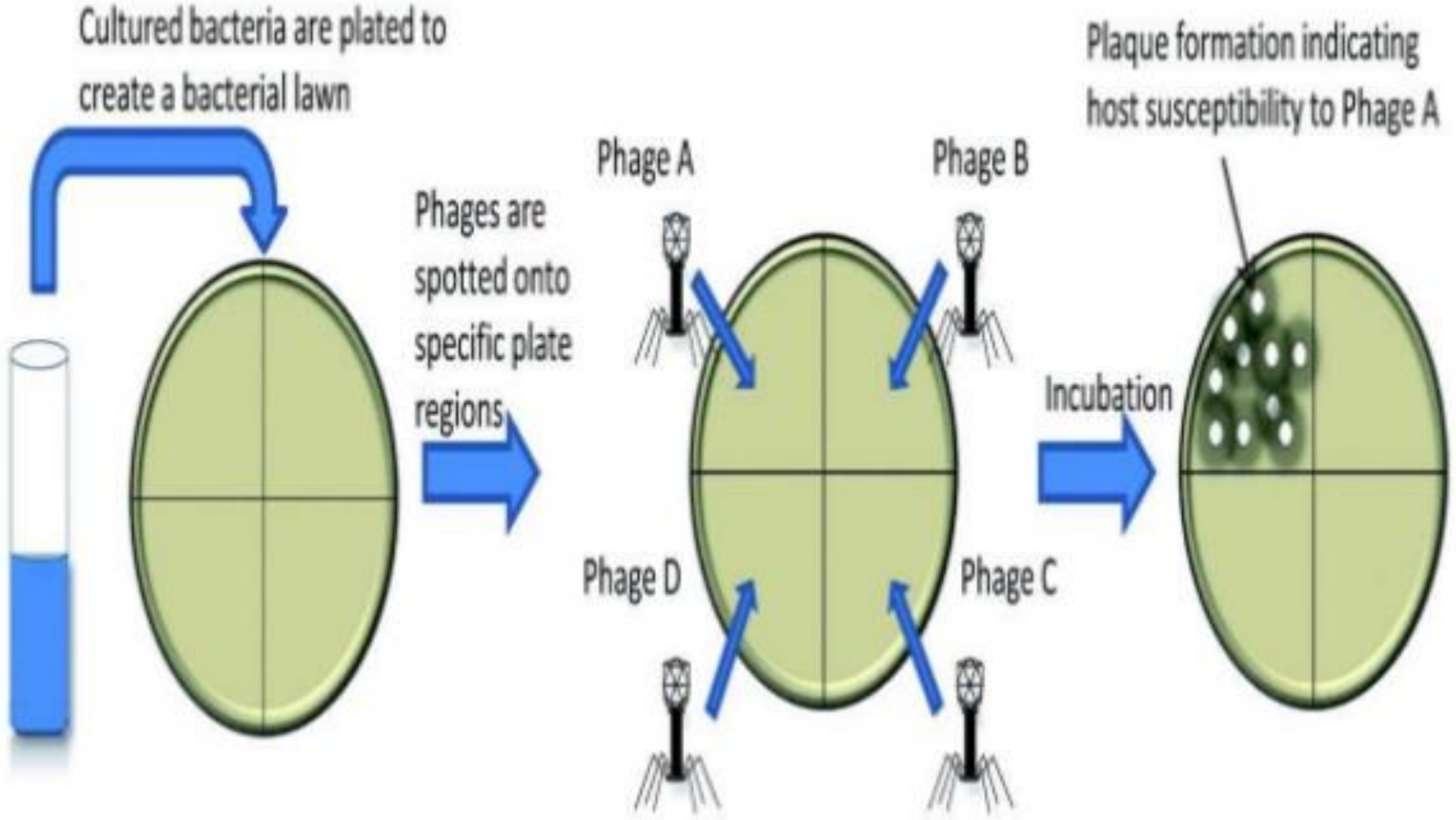
# Immune Testing

- Numerous types of serologic test -differ in their speed and sensitivity.

1. Precipitation tests
   (a) Immuno diffusion
   (b) Immunoelectrophoresis
2. Agglutination tests
3. Neutralization
4. Complement fixation
5. Immuno fluorescence
6. Radioimmunoassay (RIA)
7. Enzyme-Linked Immuno Sorbent Assay (ELISA)
8. Western Blotting

**Phage typing** is a method used for detecting single strains of bacteria. It is used to trace the source of outbreaks of infections. The viruses that infect bacteria are called bacteriophages ("phages" for short) and some of these can only infect a single strain of bacteria. These phages are used to identify different strains of bacteria within a single species.




Cultured bacteria are plated to create a bacterial lawn
Phages are spotted onto specific plate regions
Phage A
Phage B
Phage D
Phage C
Incubation
Plaque formation indicating host susceptibility to Phage A

# Genotypic methods

- The initiation of new molecular technologies in genomics is shifting traditional techniques for bacterial classification, identification, and characterization in the 21st century toward methods based on the elucidation of specific gene sequences or molecular components of a cell.
- Genotypic methods of microbe identification include the use of :
  - ✓ Nucleic acid probes
  - ✓ PCR
  - ✓ Nucleic acid sequence analysis
  - ✓ 16s rRNA analysis
  - ✓ RFLP
  - ✓ Plasmid fingerprinting.

*RFLP : restriction fragment length polymorphism

# Advantage of genotypic methods over phenotypic methods

- SPEED,ACCURRACY,COST
- ability to detect nonviable organisms that are not retrievable by cultivation based method.
- identification of bacteria grown in culture

1)Slow growing bacteria

2)Common pathogen exhibit unusual phenotypic traits.

- detection of antimicrobial resistance.

# Continue....

- characterization of bacteria beyond identification

1)For identifying virulence, resistance, strain relatedness of same species.

- ability to quantitative analysis of infectious agent burden directly in patients specimens.

** continue

- To recognize and control disease outbreak inside or outside the hospital.