Lec5: Classification, identification of bacteria& the laboratory diagnosis

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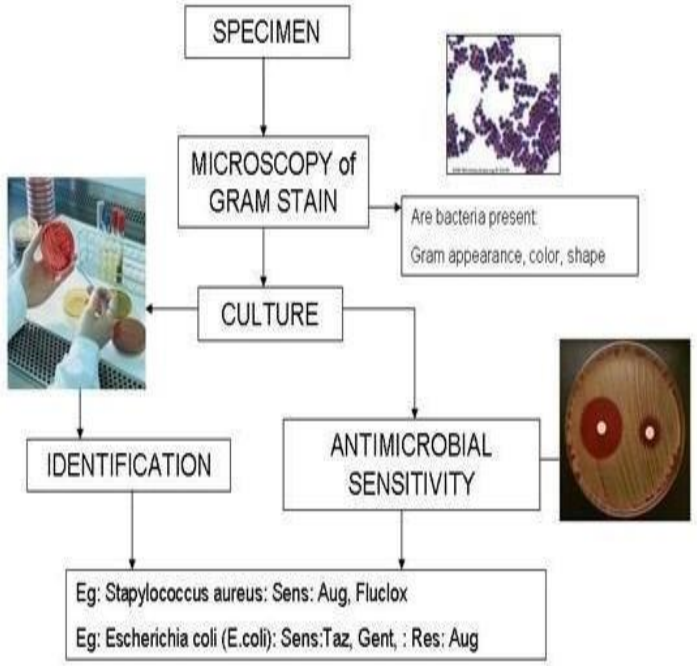
CLASSIFICATION ,IDENTIFICATION OF BACTERIAAND THE LABORATORY DIAGNOSIS

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** التسمية
- **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.

Note:
"Taxa" is a taxonomic group of any rank, such as a species, family, or class.
الاصناف.

Naming microorganisms

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

Staphylococcus aureus (S. aureus)

Bacillus subtilis (B. subtilis)

Escherichia coli (E. coli)

Taxonomic Rank

- Kingdom or Domain المملكة أو المجال
- Division or Phylum القسم
- Class الطبقة
- Order الترتيب
- Family العائلة
- Genus الجنس
- Species الانواع

} Usually we deal with those three ranks.

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**.

To use proper sterilizing techniques and to wear gloves and a lab coat as well as cleaning the area and equipments to avoid any contamination.

- Correctly obtaining the specimen.
- Correctly handling **the specimen**
- Quickly transporting the specimen to the lab.

Putting the specimen in a proper transport medium and obtaining the specimen correctly from the patient.

- 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

يتم عمل اختبارات الحساسية لمعرفة أنواع المضادات الحيوية المناسبة للتخلص من البكتيريا.

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 الأبواغ formation

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

THE METHODS USE TO IDENTIFY BACTERIA FALLINTO THREE CATEGORIES

1. Phenotypic classification: التصنيف المظهري
   - Morphology(macro -seen by naked eye- and microscopic)
   - Microscopy Gram staining characteristic **(gram (+) or gram (-)).**

- Growth requirement and metabolic behavior. (biochemical test methods)

2. Immunological (serological) tests. **By taking a serum specimen from the patient to detect antibodies or antigens. (we do not use this method with all specimens).**
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: **Immunological tests.**

5- Molecular biology techniques

6- Bacteriophage typing

Used to differentiate between different strains .السلالات

وتستخدم لتتبع مصادر انتشار العدوى

Microscopy

Microorganisms can be examined microscopically for:

a- Bacterial motility: **(to know whether the bacterium is motile or not).**

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

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 bacterialcells.

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)

What's special about this type is that the lipid content in its cell wall makes the staining process difficult.

Simple Staining Procedure:

Preparation of a smear and heat fixing

1Using a sterilized inoculating loop, transfer loopful of liquid suspension containing bacteria to a slide (clean grease free microscopic slide) or transfer an isolated colony from a culture plate to a slide with a water drop.

2 Disperse the bacteria on the loop in the drop of water on the slide and spread the drop over an area the size of a dime. It should be a thin, even smear.

3-Allow the smear to dry thoroughly.

4-Heat-fix the smear cautiously by passing the underside of the slide through the burner flame two or three times. It fixes the cell in the slide. Do not overheat the slide as it will distort the bacterial cells.

Microscope glass slide and cover slip

These are used in the hanging drop method.
The procedure:
->Drop of bacterial suspension and then we spread it over the slide, after that we examine the coverslip under the microscope.

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.

In order to have a pure colony formed.

تعريف مبدئي

تعريف نهائي

Culture Techniques

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

For example, pseudomonas aeruginosa produces a blue-green pigment. Another example is staphylococcus aureus which produces a golden pigment. (Aureus=Gold).

Hemolysis on blood agar:

That contains RBCs. Bacteria are classified here according to their ability of hemolyzing RBCs.

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

Notice here how the colonies are surrounded by a very clear zone.

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

Incomplete (partial) hemolysis, greenish color appear.

- **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

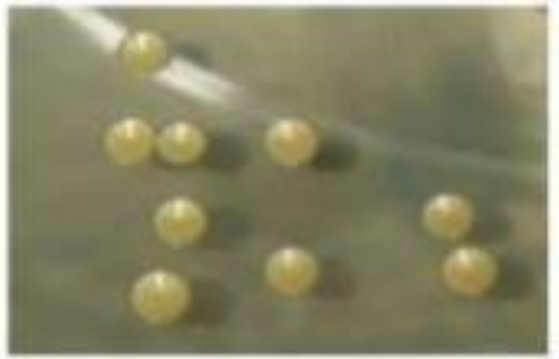
3- Selective media: allow needed bacteria to grow
. Lowenstein–Jensen medium
. MacConkey's agar
. Mannitol Salt Agar
4- Indicator media: to different. between lact. and non lact. ferment
. MacConkey's medium
. Eosine Methylene blue Agar
5- Anaerobic media: for anaerobic cultivation
. Deep agar, Robertson's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

Colonial appearance on culture media

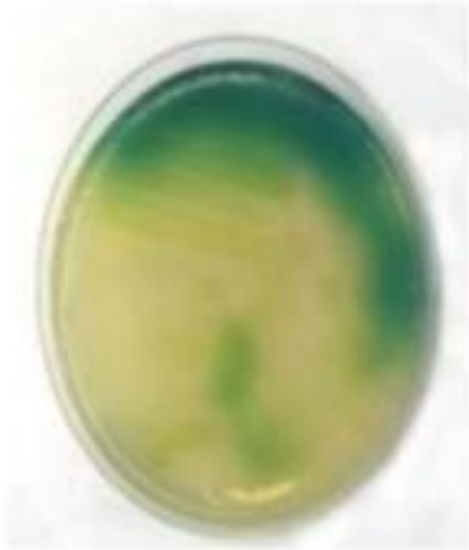
* Colony morphology:
  . Shape . Size . Edge of colony . Color
* Growth pattern in broth:
  . Uniform turbidity
  . Sediment or surface pellicle
* Pigment production:
  . Endopigment production (*Staph. aureus*)
  . Exopigment production (*Ps. aeruginosa*)
* Haemolysis on blood agar:
  . Complete haemolysis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

Motile bacteria which own a flagella can swim and their movement would create a uniform turbidity. On the other hand, non-motile bacteria with waxy cell walls tend to float at the surface of the broth producing a surface membrane called a pellicle.

- **Pigment production:** These pictures illustrate pigment production
  - **Endopigment** (restricted to the colonies): The colour of these colonies
    - Golden yellow with *Staphylococcus aureus*.
    - White with *Staph. epidermidis.*

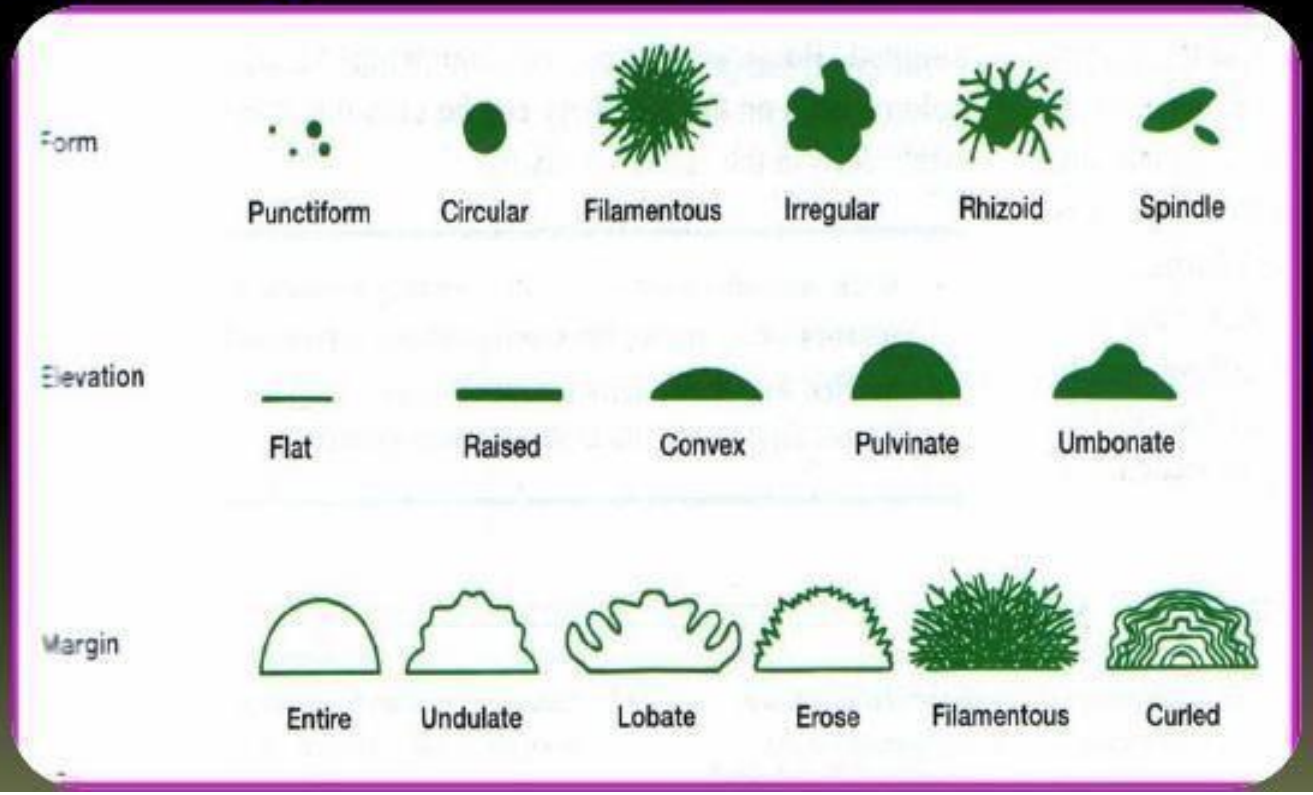


  - **Exopigment** (the color diffuses in the surrounding medium):
    - Green exopigment with *Pseudomonas aeruginosa*.



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

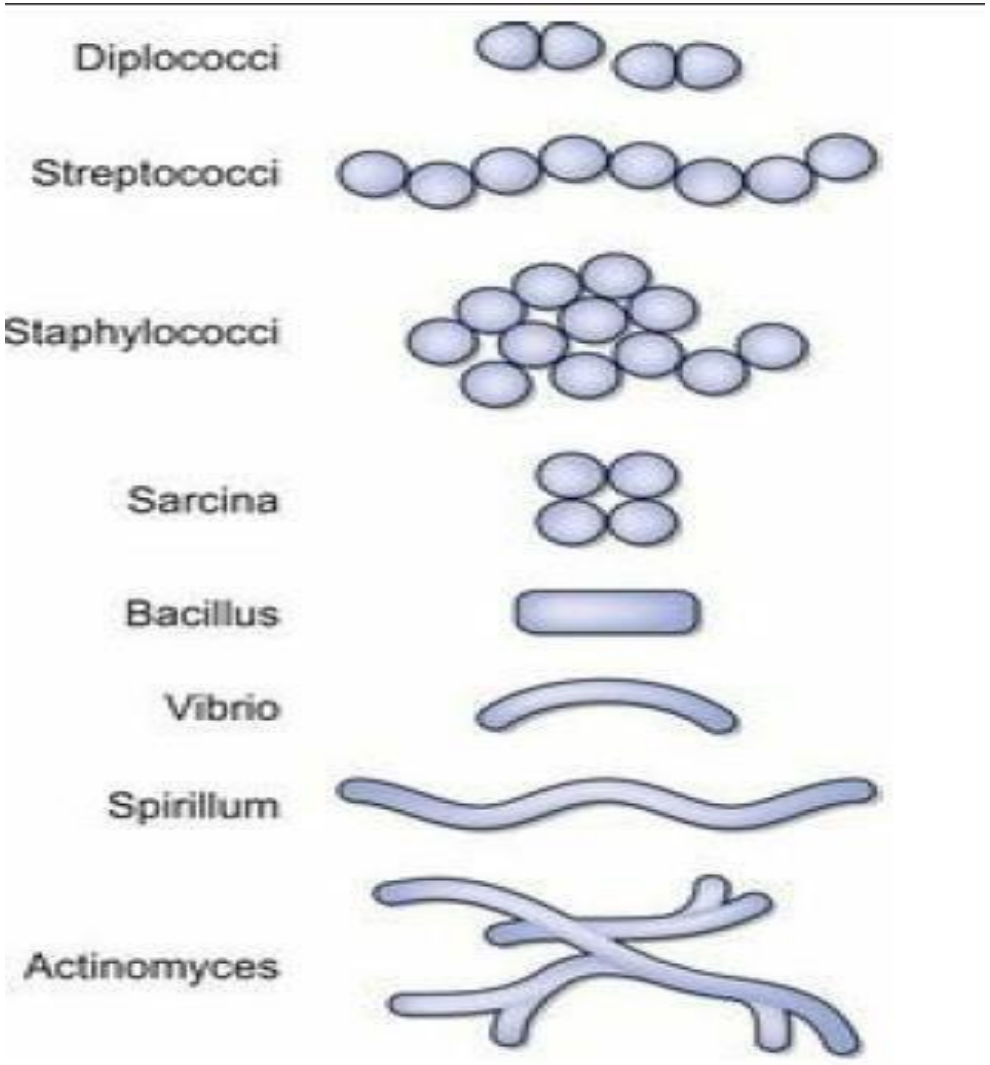
- **Colony form**: pinpoint, circular, filamentous, irregular
- **Colony elevation:** flat, raised, convex
- **Colony margin:** smooth, irregular

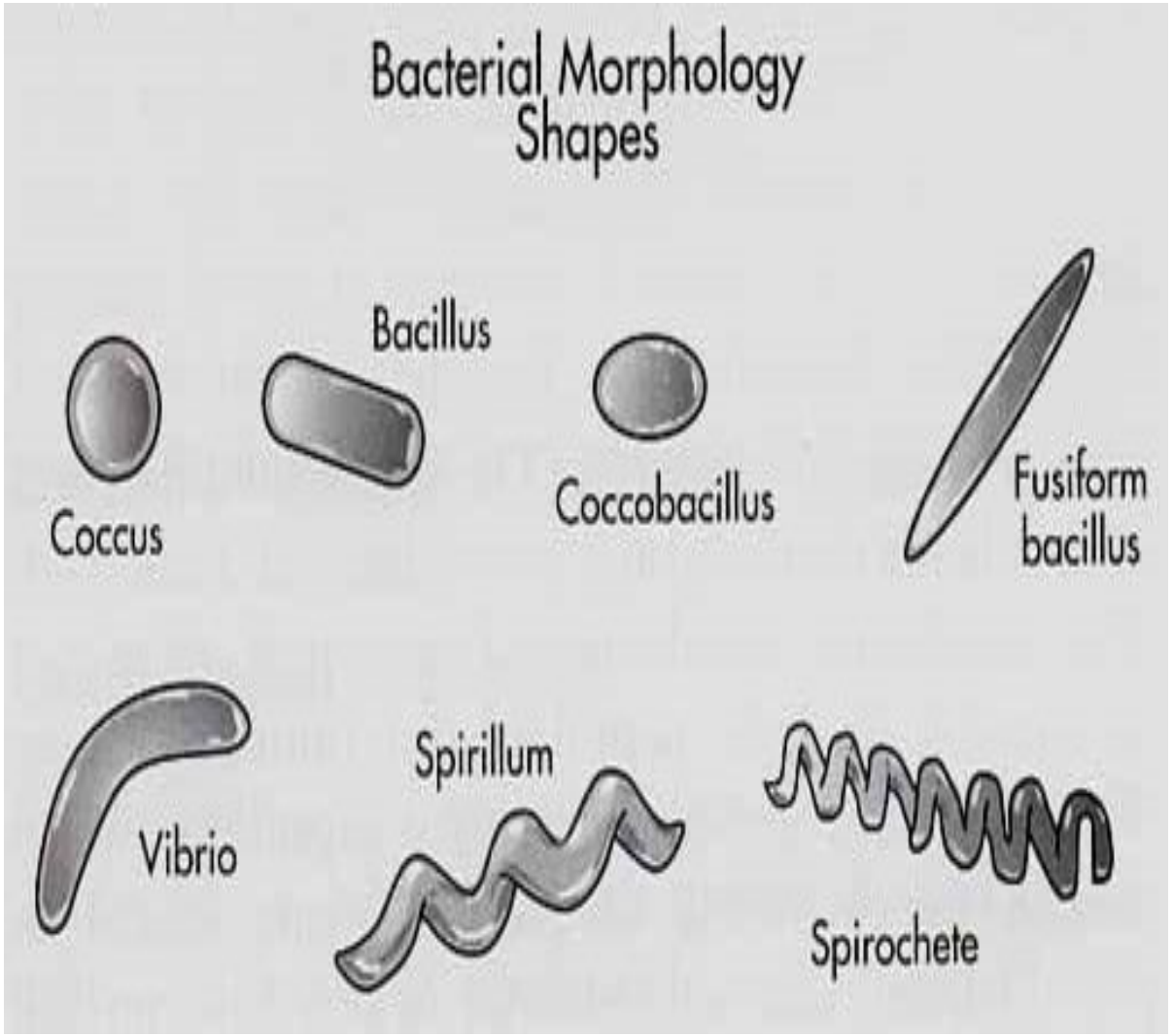


These characteristics helps in the identification of the bacteria

COLONY CHARACTERISTICS

Can’t be seen by the naked eye only under the microscope





Morphology:

- Cocci Spherical
- Bacilli Rod-like
- 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

Last part of phenotypic classification

(Use of substrates and sugars to identify pathogens)

****Sugar fermentation:**

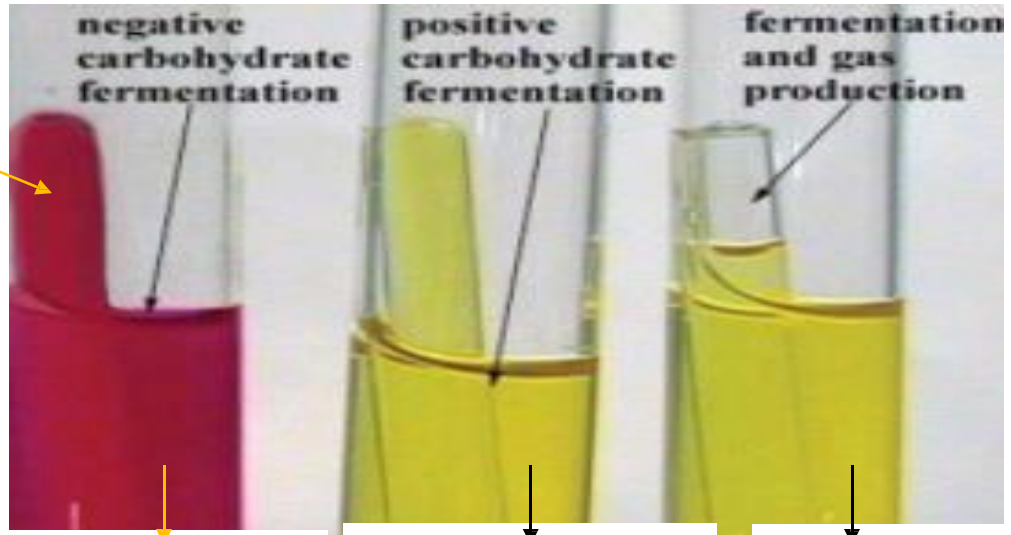
Organisms ferment sugar with production of acid only.

Organisms ferment sugar with production of acid and gas.

Organisms do not ferment sugar.

Glucose broth with Durham tubes

- This is a differential medium. It tests an organism's ability to ferment the sugar glucose as well as its ability to convert the end product of glycolysis, pyruvic acid into gaseous byproducts. This is a test commonly used when trying to identify Gram-negative enteric bacteria, all of which are glucose fermenters but only some of which



The microorganism couldn't ferment the sugar so the colour remained red

The microorganism has the ability to ferment the sugar Red-> yellow

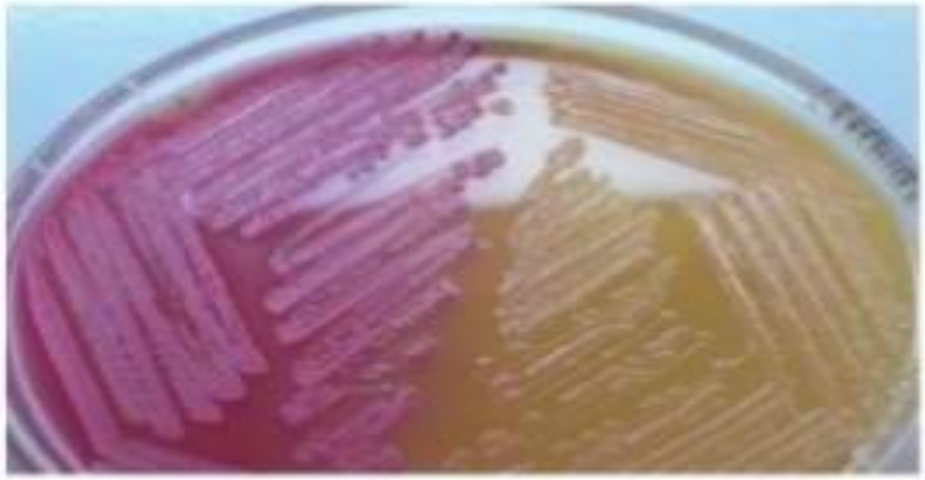
Fermentation with the production of gas (bubbles in the tube)

Effect on lactose of MacConkey's agar:

Some microorganisms can ferment the lactose in the MacConkey's agar media

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

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

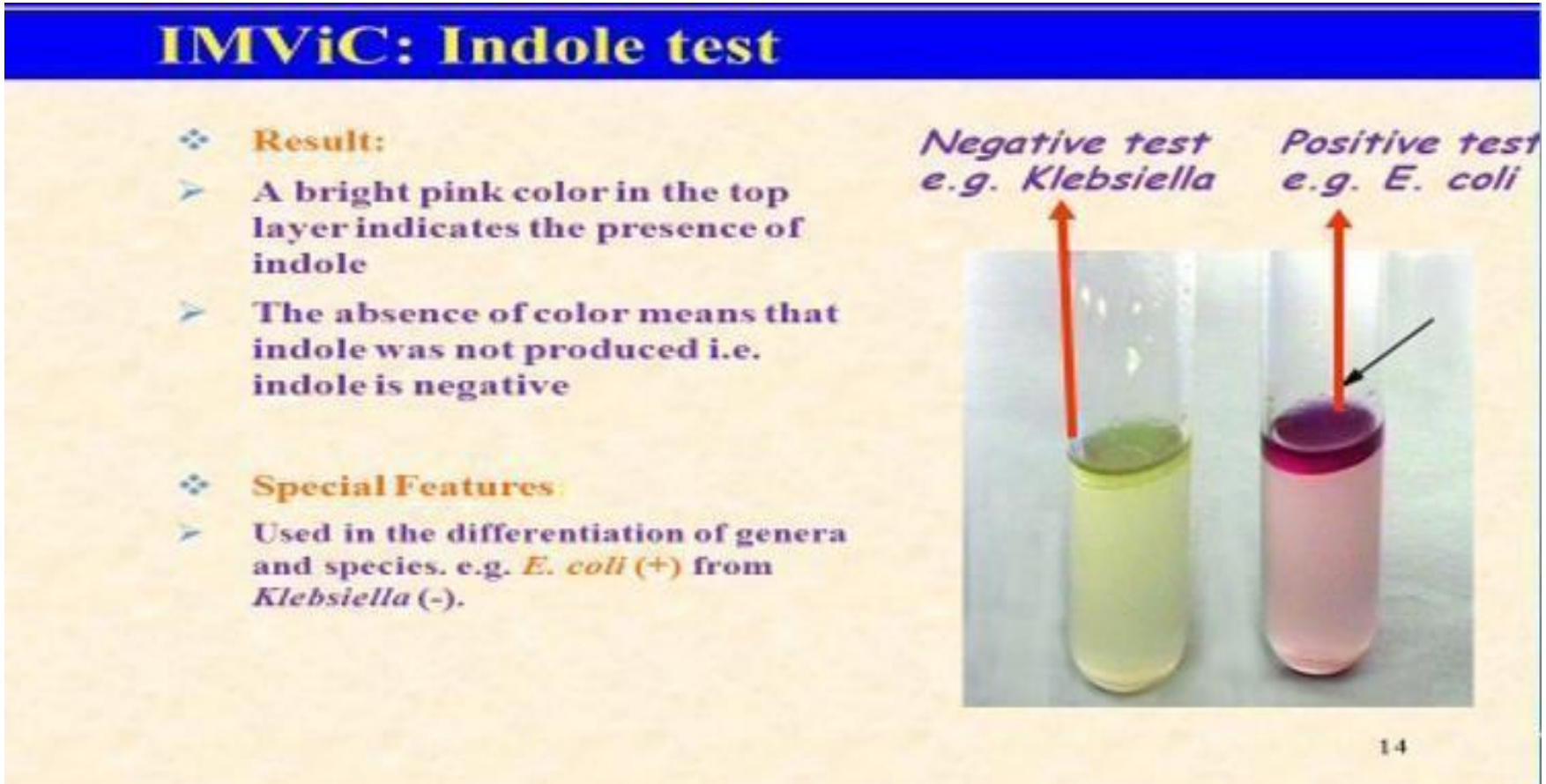


** PRODUCTION OF INDOLE :

After the previous experiment we have to differentiate between the lactose fermenters

Depends on production of indole from amino acid tryptophan . Indole is detected by addition of Kovac's reagent.

Appearance of red ring on surface.



** *HYDROGEN SULFIDE (H2S) PRODUCTION TEST*

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

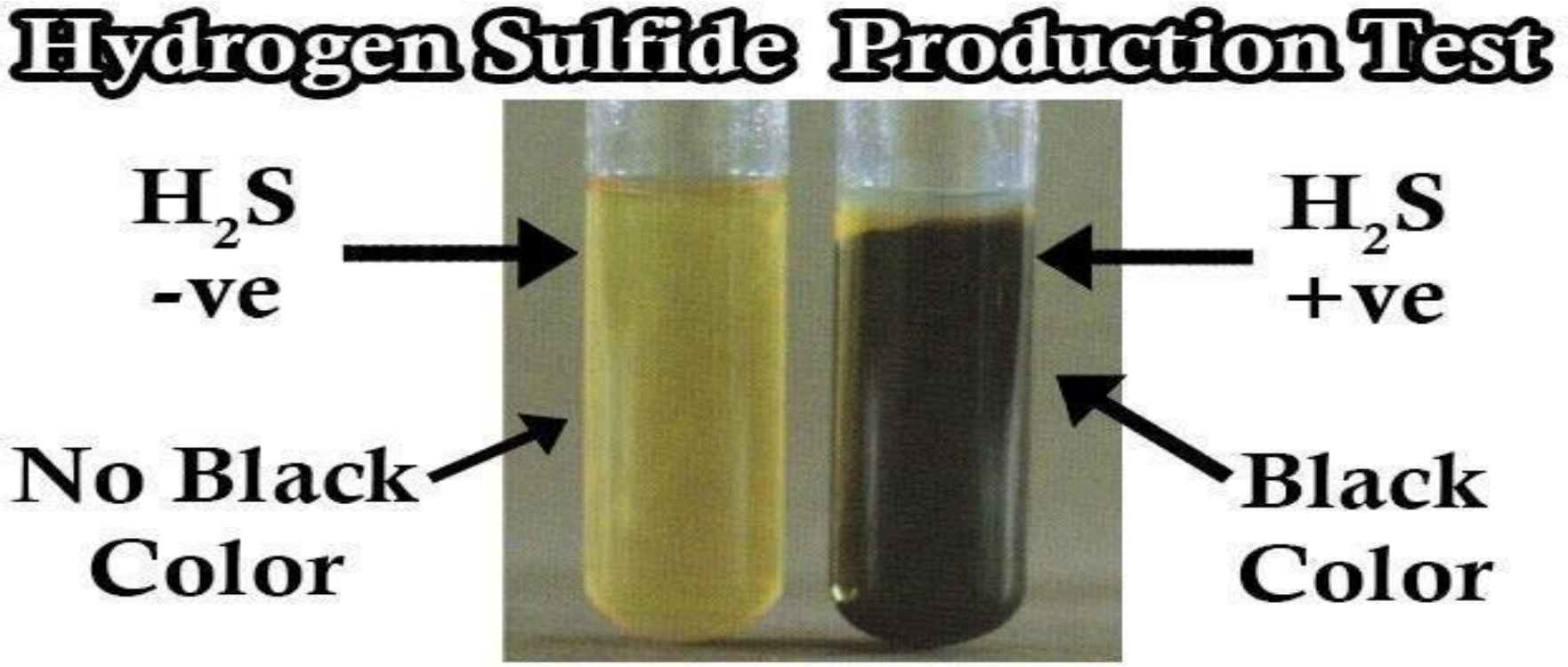
Several media containing ***iron compounds*** allow detection of hydrogen sulfide production.

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

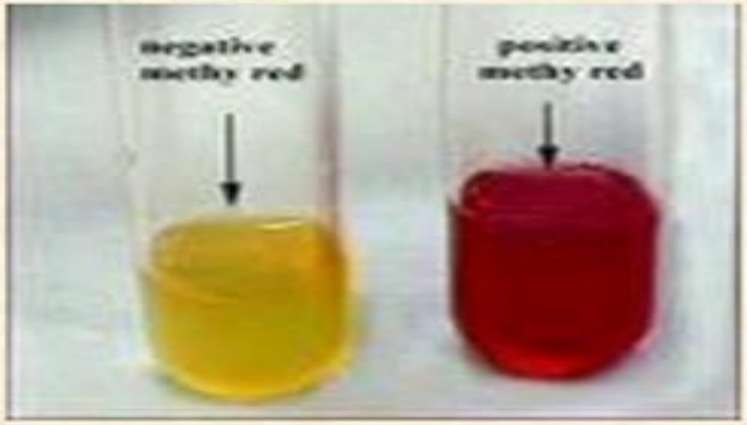
Biochemical Reaction (cont.)

- 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

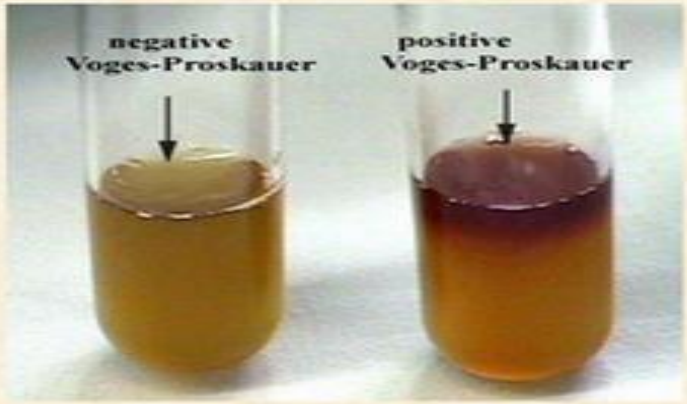
❖ *Results*



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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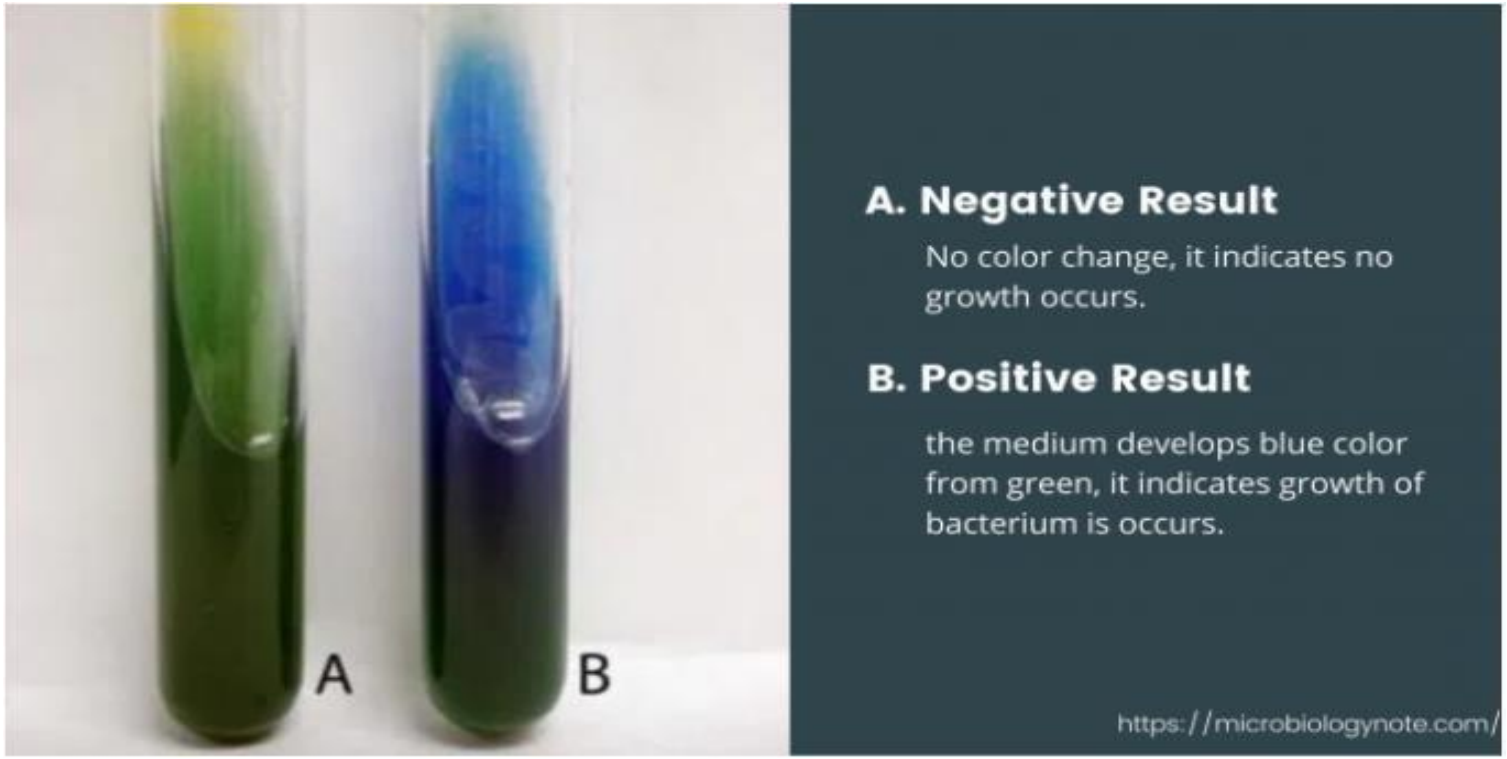
In the lab, many experiments are performed to precisely identify the bacteria

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:** Shape: cluster

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

- Principle:

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

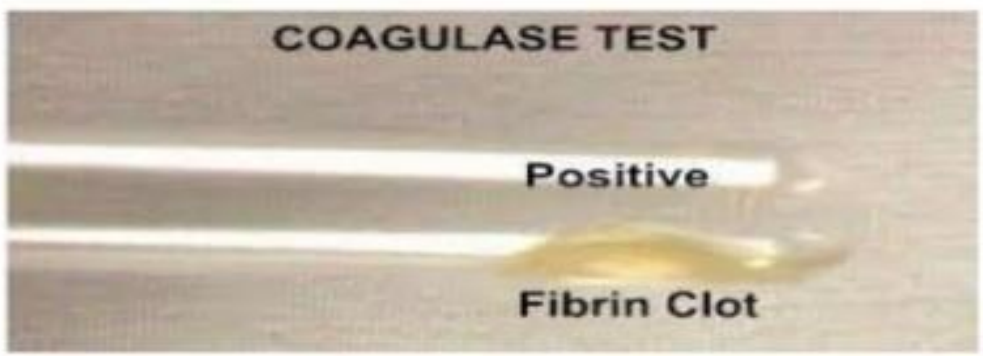
- Procedure
  - Smear a colony of the organism to a slide
  - Drop H_2O_2 onto smear
  - Observe

- 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

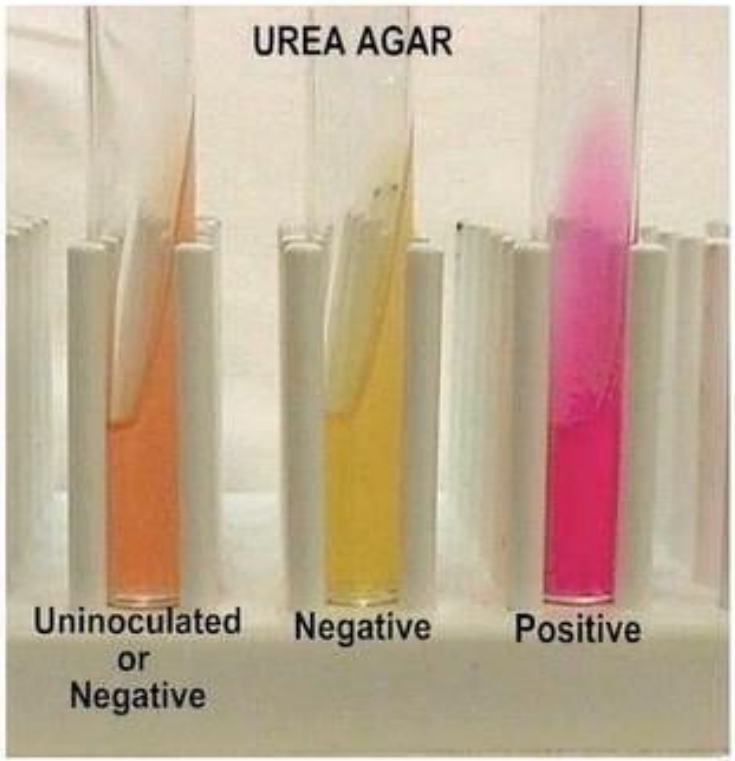
Exercise- inoculate

- E.coli, Proteus vulgais
- unknown

- Incubate 48 h at 37 ^{0}c

Interpretation:

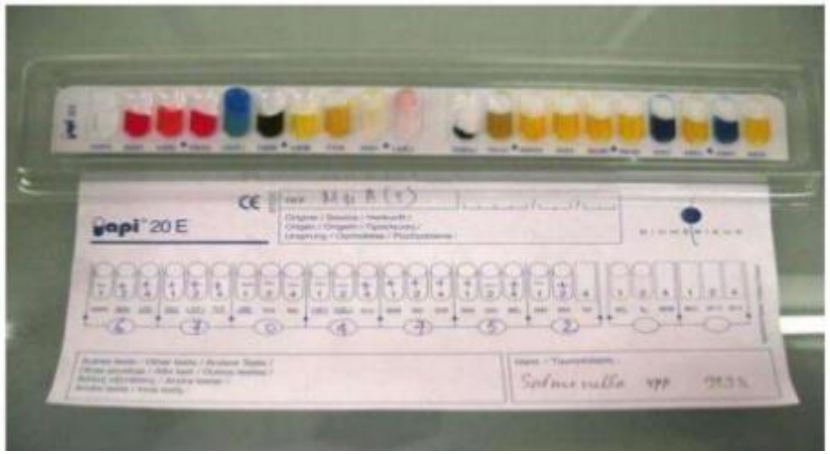
- The release of ammonia by the breakdown of urea results in the an alkaline pH of the medium which will turn the medium pink.
- Thus pink color indicates production of urease by the organisms
- Absence of pink color indicates a negative test for urease.



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.

API 20E is an example of the system
It is used to differentiate between interobacterocy (gram -).
It has cupules that contain the substrate
We put in every cupule one type of microorganisms and we detect the enzymatic activity and fermentation of sugars in the next day.
-With this method, the identification of bacteria become easier.

بدل ما أحضر الوسط لكل نوع وأستنى بعملهم كلهم مع بعض مرة وحدة

Automated bacterial identification systems:

Automated bacterial identification systems make the life easier.
Here in our example (Vitek system), we will save time and effort because no need to prepare media or do sensitivity tests.

- **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.

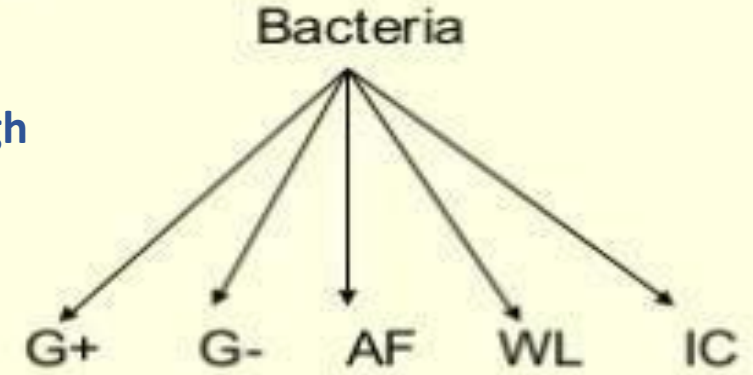
- Results are available within 4-6 hours.

Vitek system

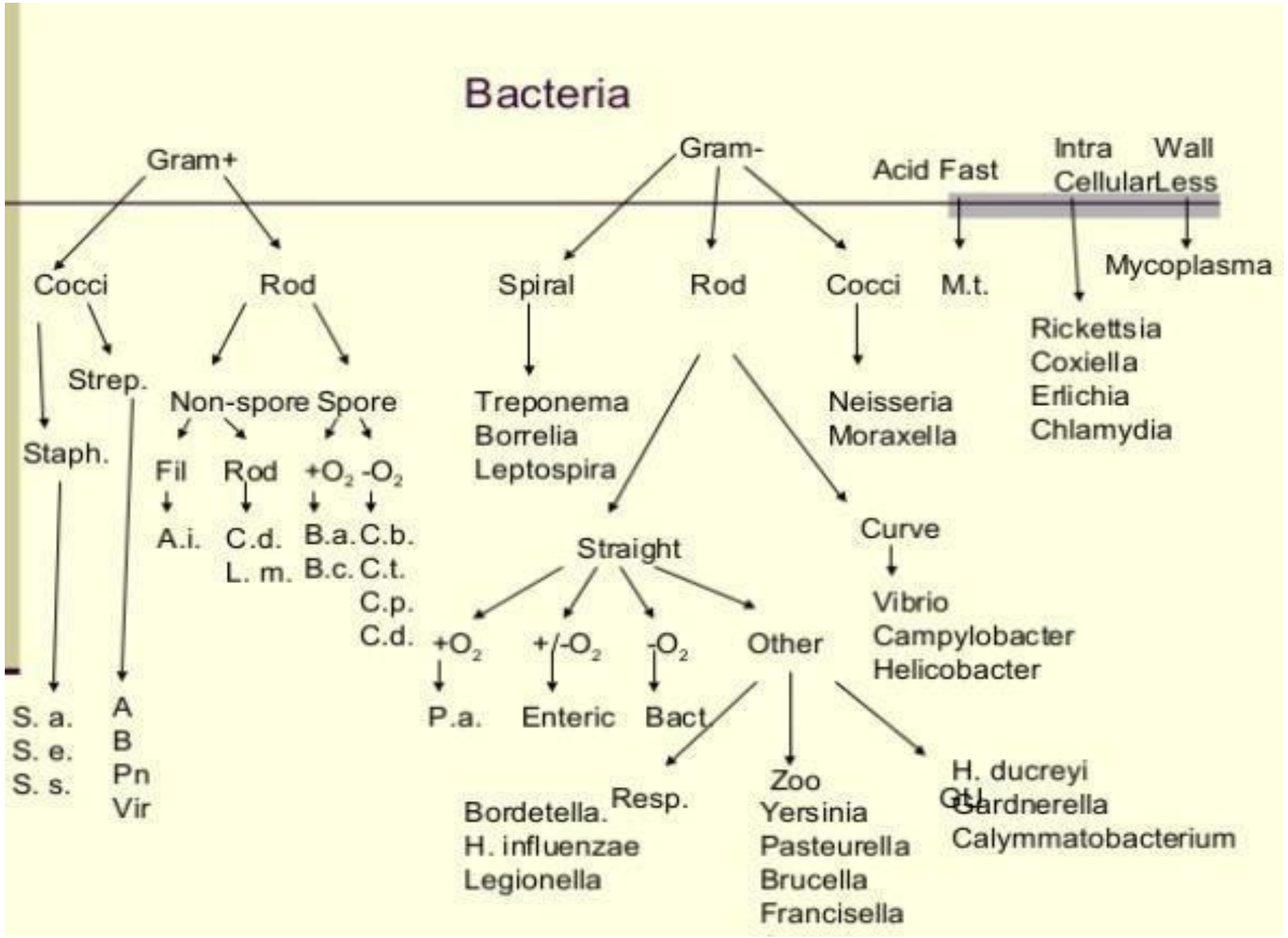
Vitek card

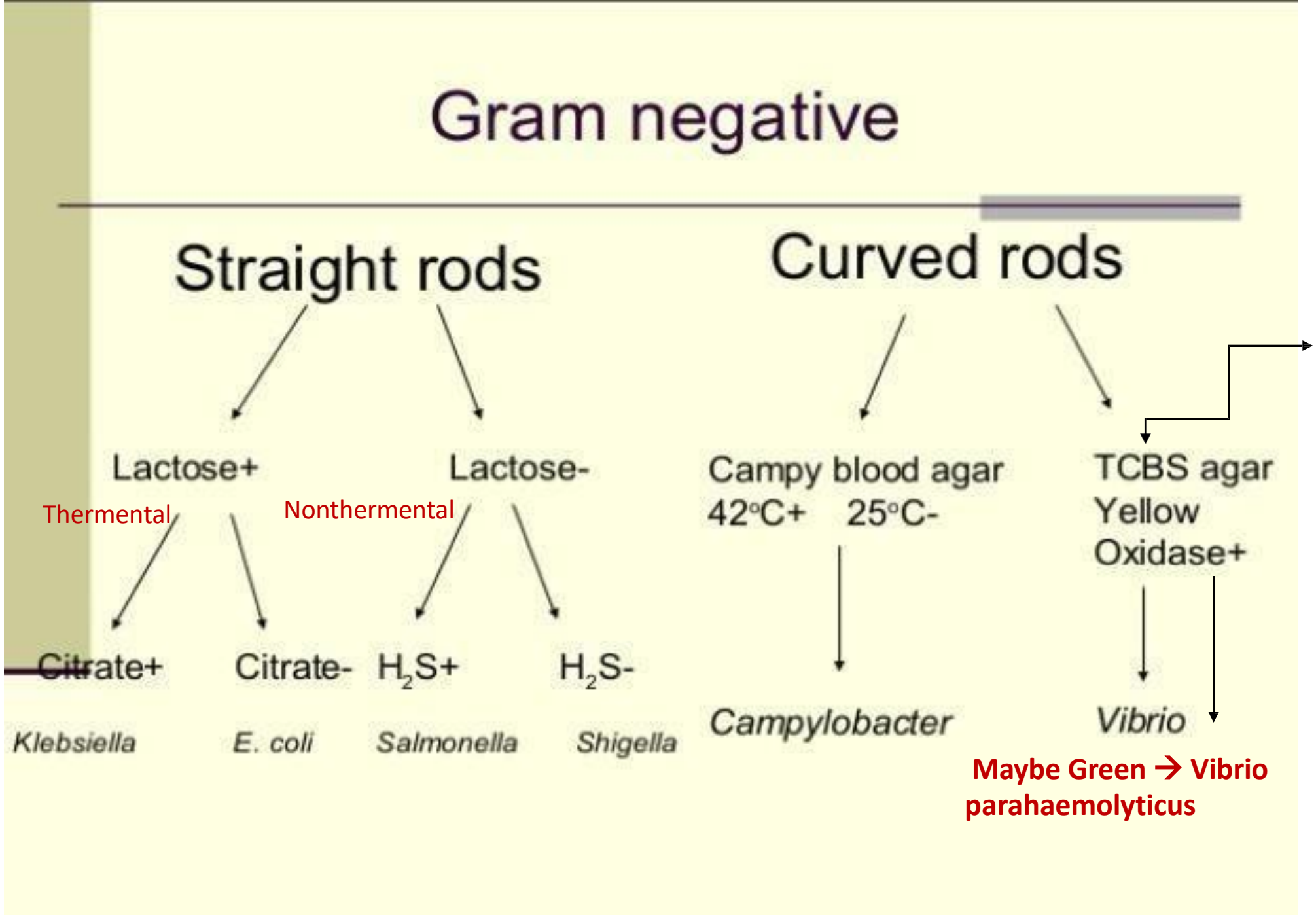
Bacteria are of many types

- With Cell Wall
  - Gram +
    - *Staphylococcus*, *Streptococcus*, *Clostridium*, *Bacillus*
  - Gram -
    - Enteric, respiratory and others
  - Acid-fast
    - *Mycobacterium* **Lipid content is high**
  - Wall-less
    - *Mycoplasma*
- Unusual
  - Obligate intracellular **Can’t live without cells**
    - *Rickettsia*, *Chlamydia*



You **should** **memorize** these types of bacteria, and we will get it later on.




Gram negative
Straight rods
Curved rods
Lactose+
Lactose-
Thermental
Nonthermental
Citrate+
Citrate-
H2S+
H2S-
Klebsiella
E. coli
Salmonella
Shigella
Campy blood agar
42°C+ 25°C-
Campylobacter
TCBS agar
Yellow
Oxidase+
Vibrio
Thiosulfate
citrate bile
salts
sucrose
Maybe Green → Vibrio
parahaemolyticus

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.

We use animals for:

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

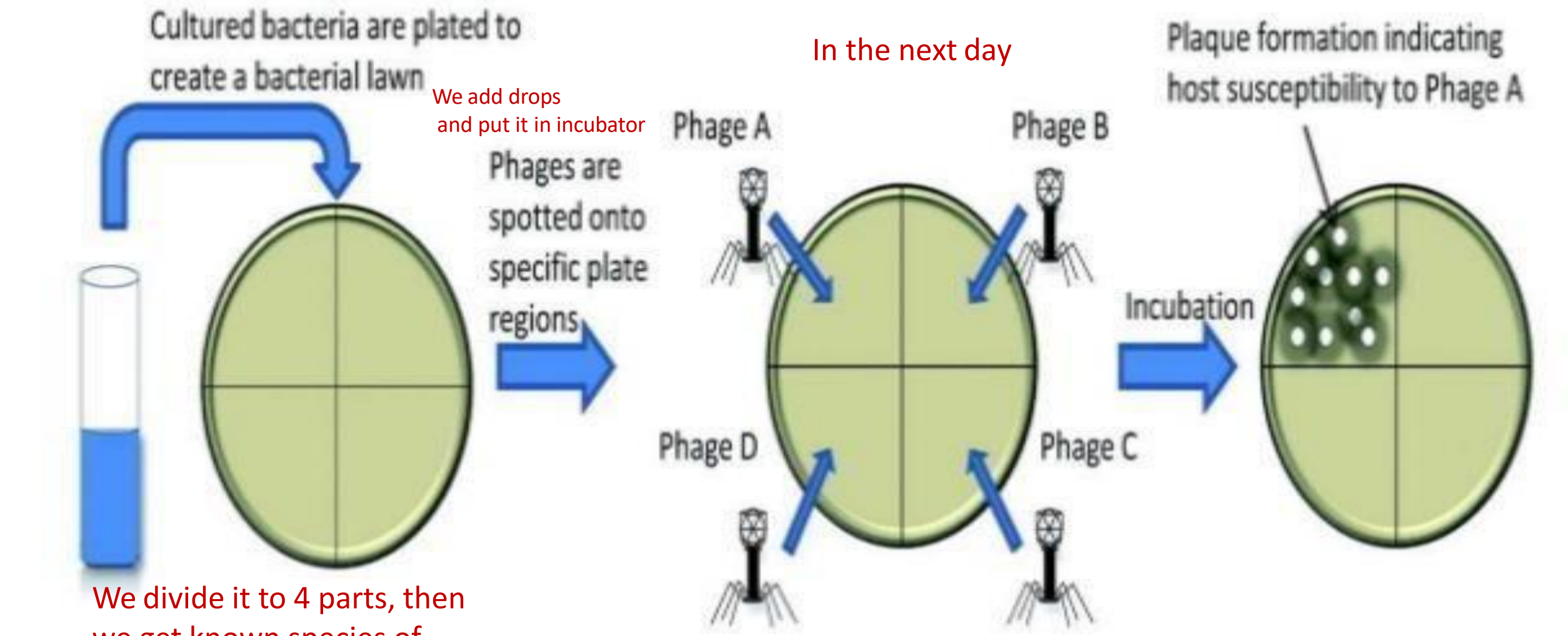
- **Numerous types of serologic test -differ in their speed and sensitivity.**

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 bacteriaare 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.

Bacteriophage typing

- Bacteriophages are viruses which infect the bacterial cells and cause their lysis.
- Different types of a certain bacteria are lysed by different phage groups.
- If a phage is added to a plate inoculated with susceptible bacteria, a zone of lysis will appear around the phage drop.



We divide it to 4 parts, then we get known species of phages

So we know and identify for bacteria

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.