MICROBIOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 4

Bacterial Taxonomy, Classification, and Laboratory Diagnosis (Pt.1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

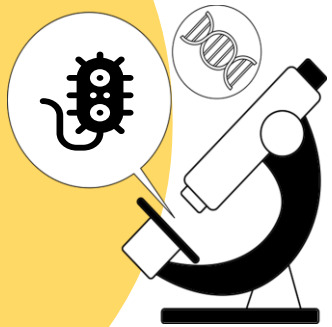
اللهم استعملنا ولا تستبدلنا

Written by:

- Mohammed Alshwyiat
- Khaled AlKhandaq
- Bashar Khraisat

Reviewed by:

- Ahmad Abu Aisha
- Muthanna Khalil



Objectives

Def. of Taxonomy

Nomenclature

Scheme of medical bacteria

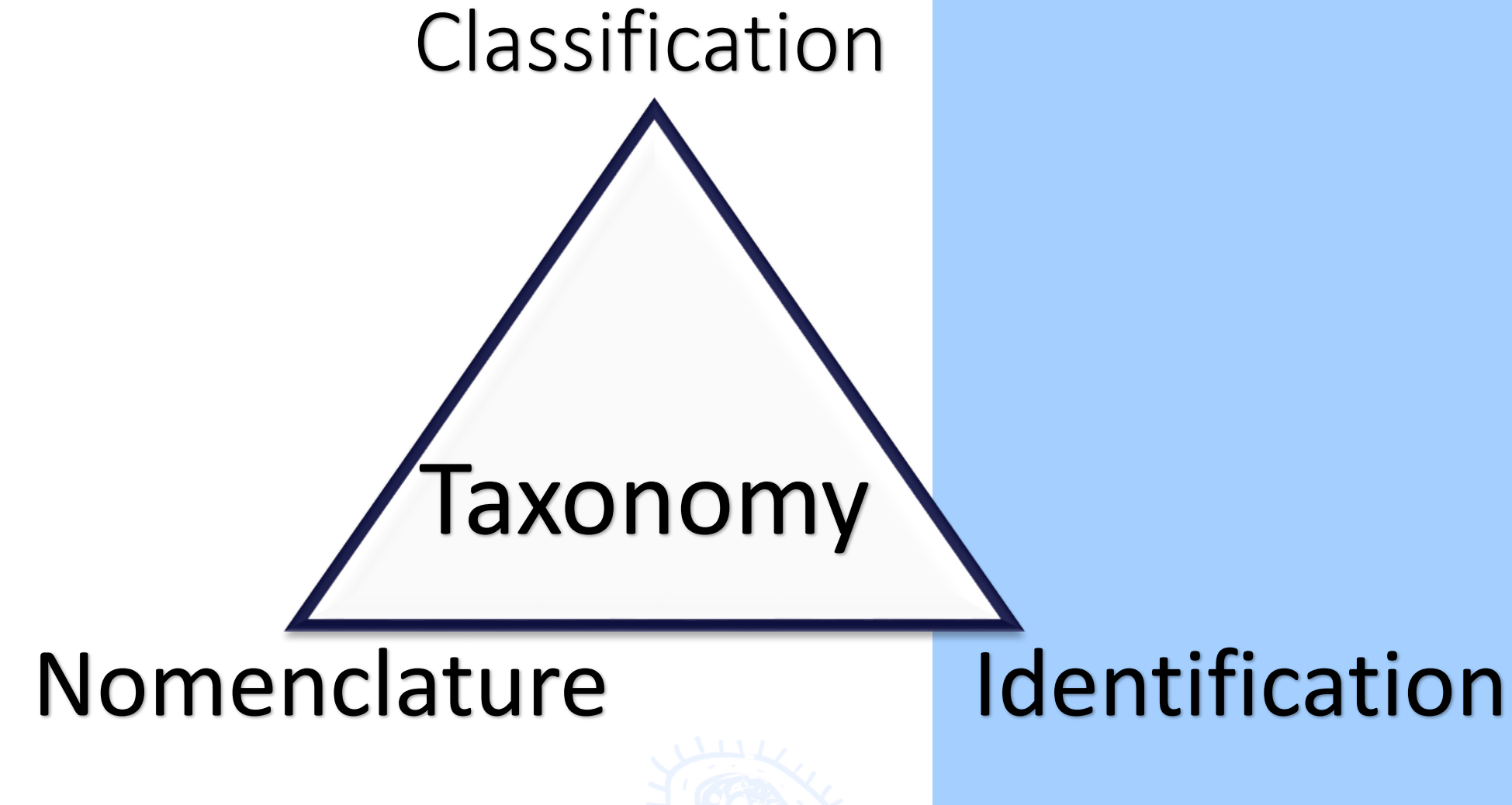
Biochemical reactions

Bacterial Taxonomy

Taxon= group , Taxa= groups = classification

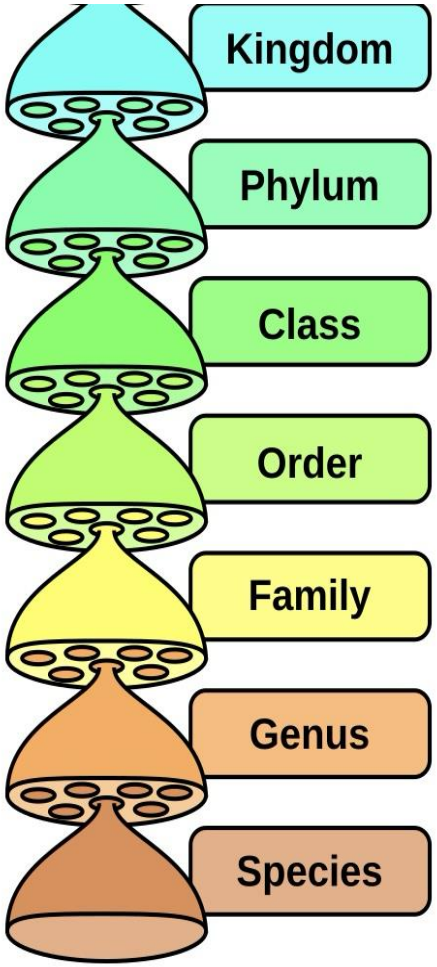
The science of biological classification

Bacterial Taxonomy

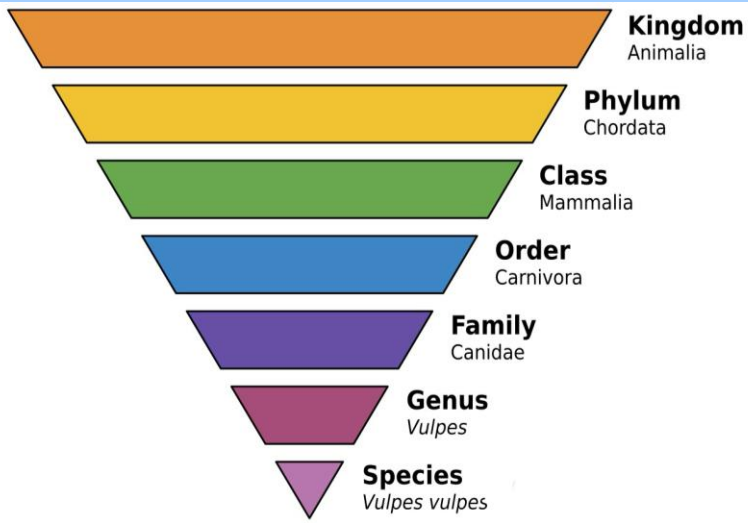


Bacterial Taxonomy Rank

- Kingdom or Domain
- Division or Phylum
- Class
- Order
- Family
- Genus — Plural is genera
- Species — Plural is species
- Strains



A kingdom consists of multiple divisions, a division of multiple classes, a class of multiple orders, an order of multiple families, a family of multiple genera, a genus of multiple species, a species of multiple strains.

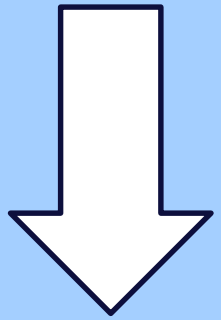


Bacterial Taxonomy

Strain

Individual member within a species

Staph. aureus
(Species)



MRSA Methicillin-resistant Staphylococcus aureus
(Strain)

Staph. aureus is a species that consists of many strains, MRSA is one of the many strains under this species

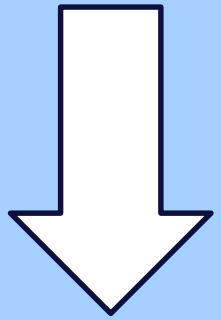
Bacterial Taxonomy

Species

A collection of strains
that share many
stable properties

Such as: Genetic and morphological properties.

Staph. aureus
(Species)



MRSA
(Strain)

VRSA
(Strain)

vancomycin-resistant
staphylococcus aureus

Bacterial Taxonomy

Species

S. aureus (Species)

Same species (DNA homology ≥ 70%)

(16S rRNA >97% identical)

species contain multiple Strains.
Bacteria are considered within the same species if these two conditions are met:
1-the DNA in these strains is least 70% identical(using DDH technique).
2-the ribosomal RNA is at least 97% identical.

Q: Two strains, the DNA is 69% identical, can we classify them as the same species?
ABSOLUTELY NOT

Bacterial Taxonomy

Genus

Staphylococci

Species

S. epidermidis

Species

S. aureus

A Genus is defined as:

One or more species

that share common properties

DNA < 93% new genus

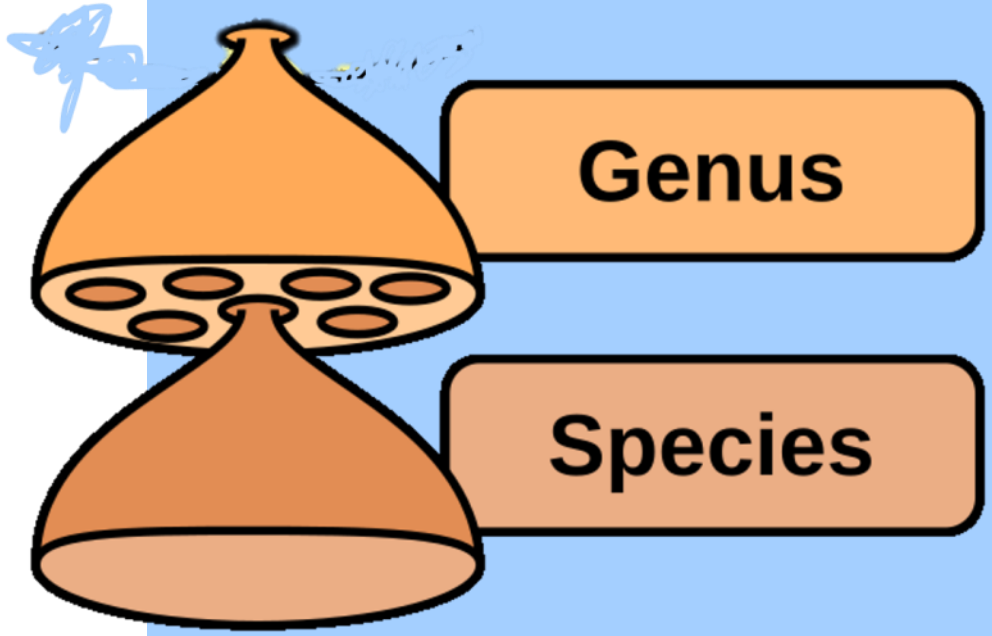
Bacteria are classified within the same genus, if they have at least 93% DNA homology (using ANI technique)

A question may arise :
how come that the limit of DNA homology is higher for genera, considering that the genus is the wider group who include different species within it?
Simply, two different techniques were used.
If we used ANI technique for the species criteria
the limit would be >95% of homology

Nomenclature

First rule of nomenclature: a name must consist of 2 words, the first word is the genus, the second is the species

Genus + species



Nomenclature

Genus

Species

Escherichia coli

first letter of genus name should always be capitalized

The species should be written all with small letters

The name should be written in *italic* or <u>underlined</u> (an old way)

<u>تحته خط</u> *بالعربي يعني مايل أو*

(E. coli)

Using the short cut this way is also permitted:
(FIRST LETTER OF GENUS NAME. species name)

I

Scheme of medical bacteria

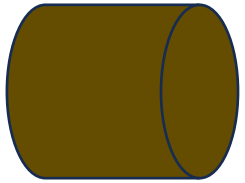
Shape

Cocci

Coccus, spherical

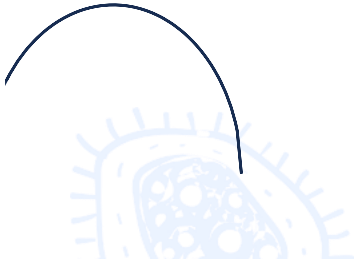
Bacilli

Bacillus, rod shaped



Spiral

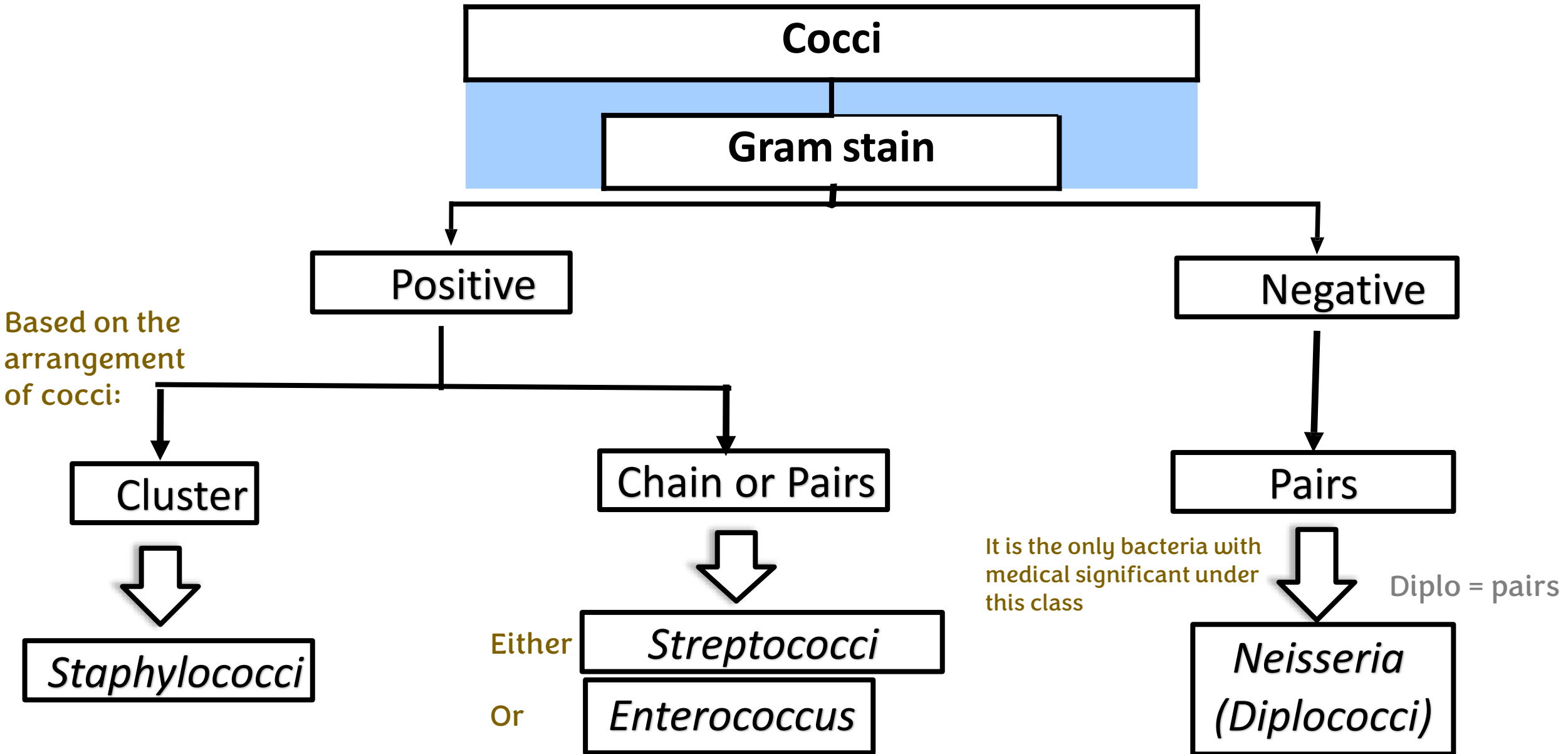
Spirillum, helical shaped



Miscellaneous

2 or more shapes

Scheme of medical bacteria



Bacilli 3

Note: Follow the numbers when describing a bacteria:

1 Gram stain

Not stain

ZN stain (TB)

It can't be gram stained, so we use a special stain called ZN, commonly used for a type called mycobacterium, the bacteria responsible for tuberculosis (TB)

2

Positive

Negative

Non-spore

4

Spore

Aerobic

5

Anaerobic

Aerobic

Anaerobic

C. Diphtheriae

Listeria

Lactobacilli

Bacillus

Clostridium

Lactobacilli is a gram[1] positive[2] bacilli[3] non spore forming[4] anaerobic[5]

Gram negative bacilli

- *Enterobacteriaceae*
- *Vibrio*
- *Campylobacter*
- *Helicobacter*
- *Pseudomonas*
- *Haemophilus*
- *Bordetella*
- *Brucella*
- *Legionella*
- Gram -ve anaerobes

Spiral

- *Treponema*
- *Borrelia*
- *Leptospira*

Miscellaneous group

- No cell wall
- Not stain by gram
- Obligate intracellular

- Mycoplasma
- Chlamydia
- Rickettsia
- Coxiella
- Actinomycetes

Systematic Bacteriology

We will discuss systematic bacteria (bacteria related to human body systems) later on, based on the following criteria

Morphology & Culture

Virulence factor&Pathogenesis

Diseases

Lab Diagnosis

Treatment & Prevention

Biochemical reactions

1) Indole test

Bacteria → Peptone (TRYPTOPHAN)

break down (Tryptophanase)

Indole

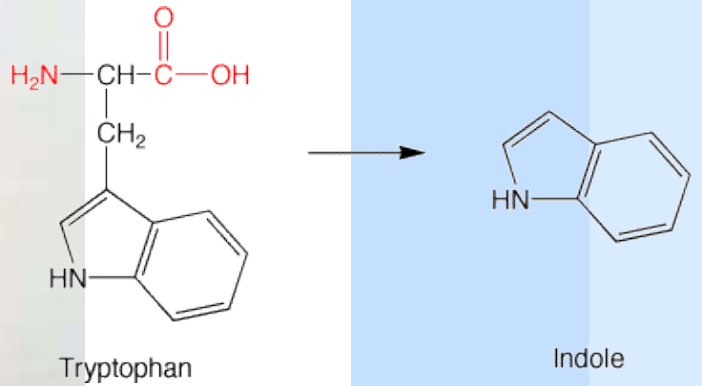
+

Kovac's R.

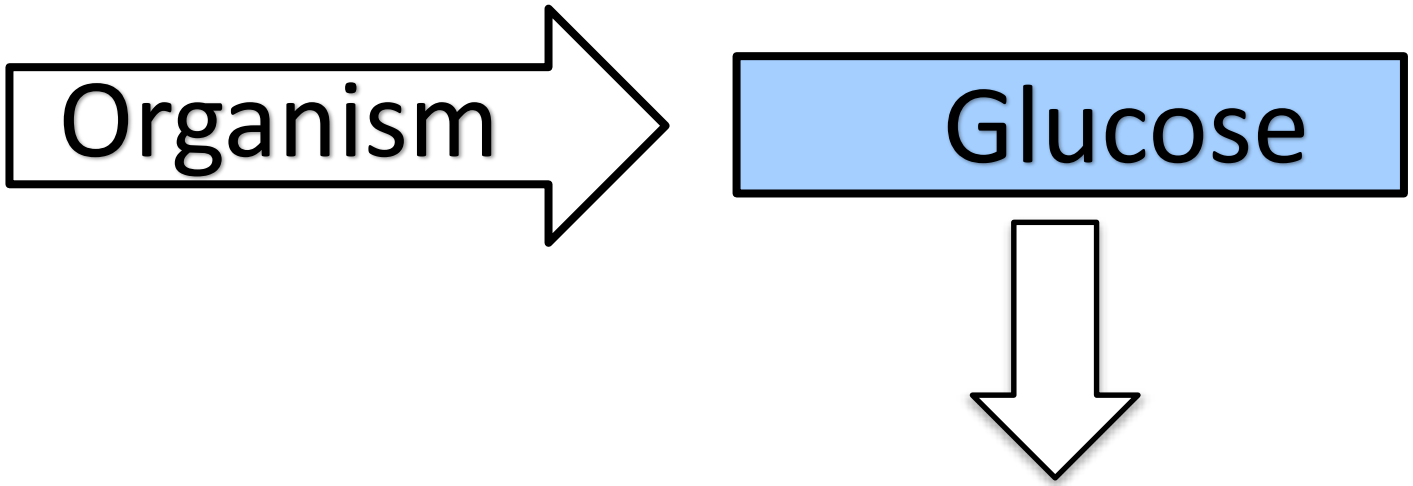
Red

- **Tests for:** bacterium's possession of Tryptophanase.
- **How?** by breaking down tryptophan in the medium and produce indole which reacts with Kovac's reagent.
- **Indication (color change) :** a red color indicates a positive result.

*Note: The medium is liquid and contains peptone (as a nutrient source) and tryptophan



2) Methyl red test



Large amount of Mixed acid
(Acetic, lactic, & succinic)

Low pH

<4

Methyl red indicator

High pH

6

- **Tests for:**

Whether bacteria can ferment glucose and produce a mixture of **acids**, such as acetic acid, lactic acid, and succinic acid.

- **How does the test work?**

By sensing the pH (acidity due to fermentation of glucose into acids) changes in the medium after the bacteria are incubated.

- **Indication (color change) :**

Methyl red indicator is added to the medium.

- If the bacteria produces the acidity required to make the pH < 4, indicator turns red, indicating an MR-positive result.
- However, if the bacteria do not produce enough acid, the medium becomes alkaline, with a pH above 6, and the color changes to yellow, indicating an MR-negative result.

Not any pH > 6 is really alkaline, but we mean less acidic and more into neutral pH.

2) Methyl red test

Bacteria → Peptone (Glucose)

↓

Incubate at 37°C for 48h

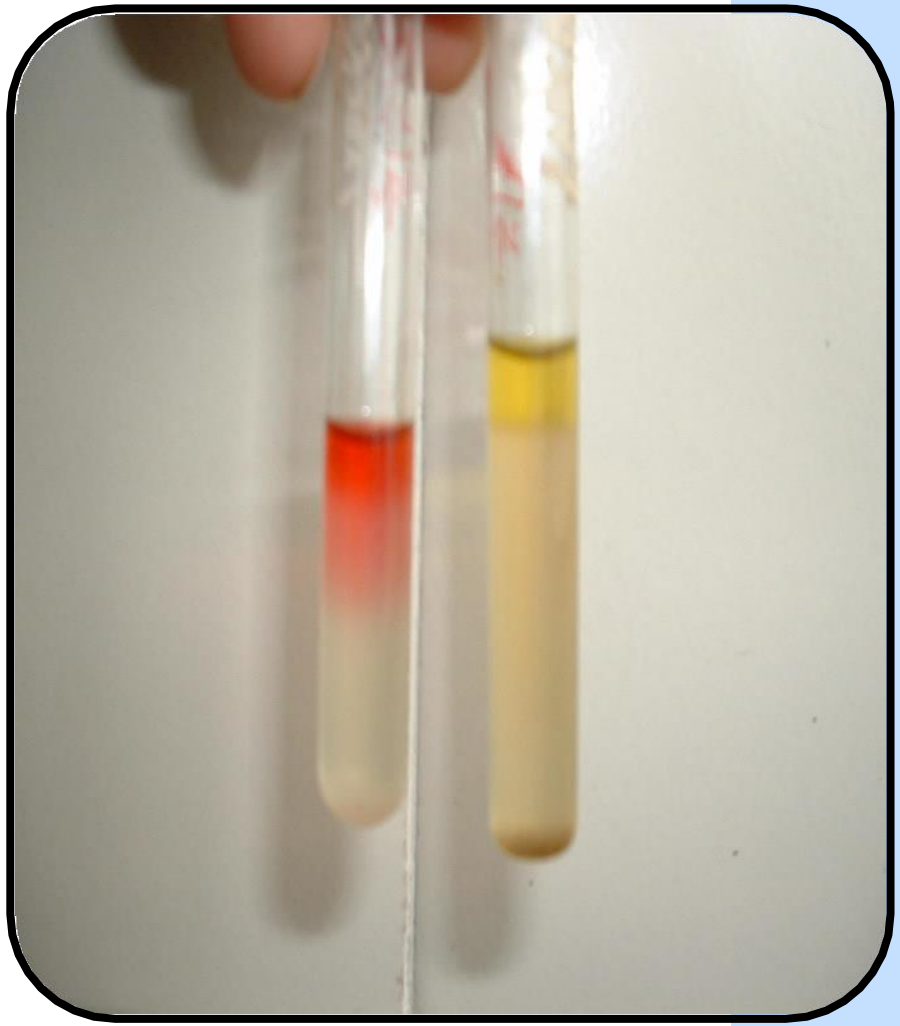
↓

(Large Acid) Mixed pH

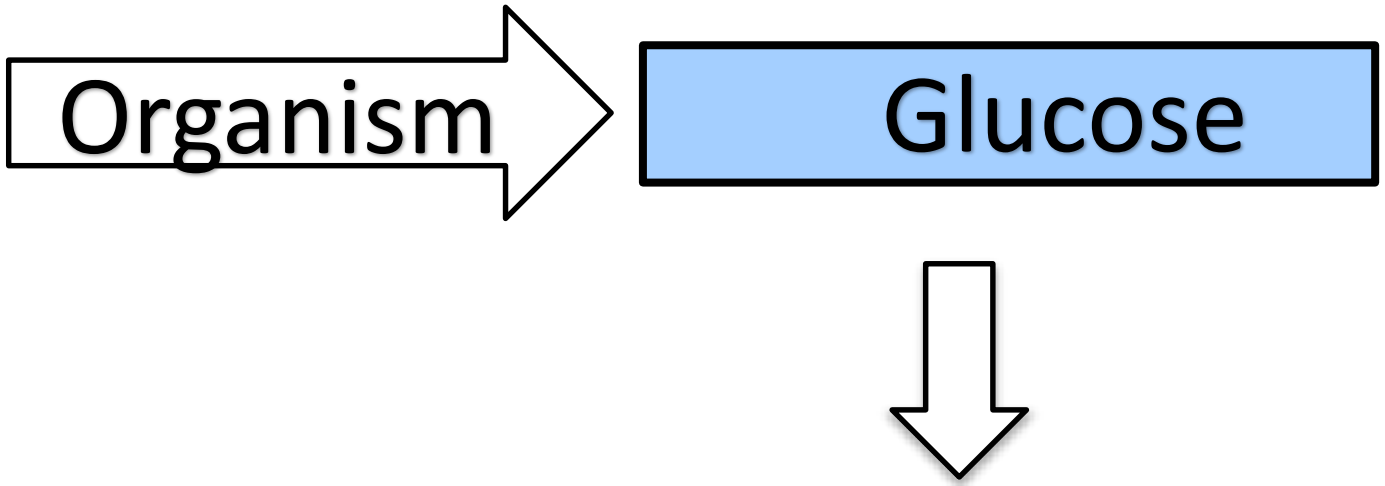
Acetic, Lactic, and Succinic acids (examples said)

+ MR indicator

MR-positive → RED color



3) Voges-Proskauer test (V.P)



Acetyl methyl carbinol (Acetoin)

- **Tests for:** the ability of bacteria to ferment glucose, producing acetoin (acetyl methyl carbinol).
- **How?**

  1. glucose is added, it would be fermented to acetoin if the bacteria have the ability
  2. alpha-naphthol and 40% potassium hydroxide (KOH)are added. When they are added, if acetoin is present, it gets oxidized to diacetyl, which reacts with peptone in the medium.
- **Indication:**

  a red color, indicating a V.P.-positive result.

3) Voges-Proskauer test (V.P)

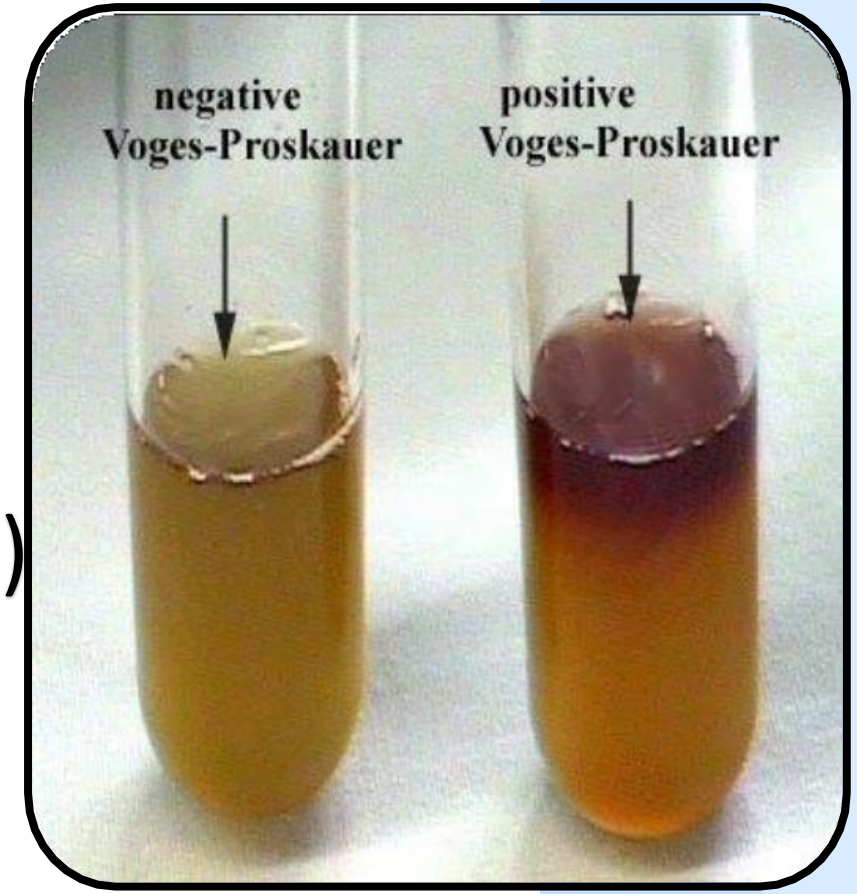
Bacteria → Peptone (Glucose)

→ Incubate at 37°C for 48h

→ Ferment glucose (Acetyl methyl carbinol)

→ a- naphthol + 40% KOH

→ Diacetyl → Red



MR &VP

If methyl red is positive, the voges-proskauer should be negative and reverse is right.

If the bacteria ferment glucose and produce acid, they typically do not produce acetoin, and vice versa.

4) Citrate utilization test

Utilized citrate as only source of carbon

- **Tests for:**

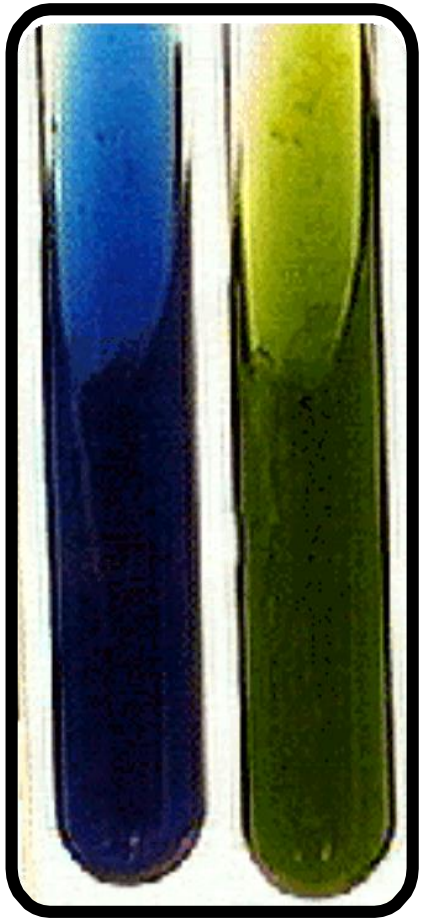
Presence of citrase (citrate lyase) in the bacteria (enzyme that breaks down citrate), or in other words, the ability of the bacteria to use citrate as a carbon source.

- **How does the test work?**

By leaving the bacteria in a medium which has citrate as the only carbon source. If citrase is present, citrate will be metabolized, releasing CO_2 which combines with Na^+ forming Na_2CO_3 (sodium carbonate) which increases pH of the medium. If no citrase is present, nothing happens, and the pH stays close to neutral.

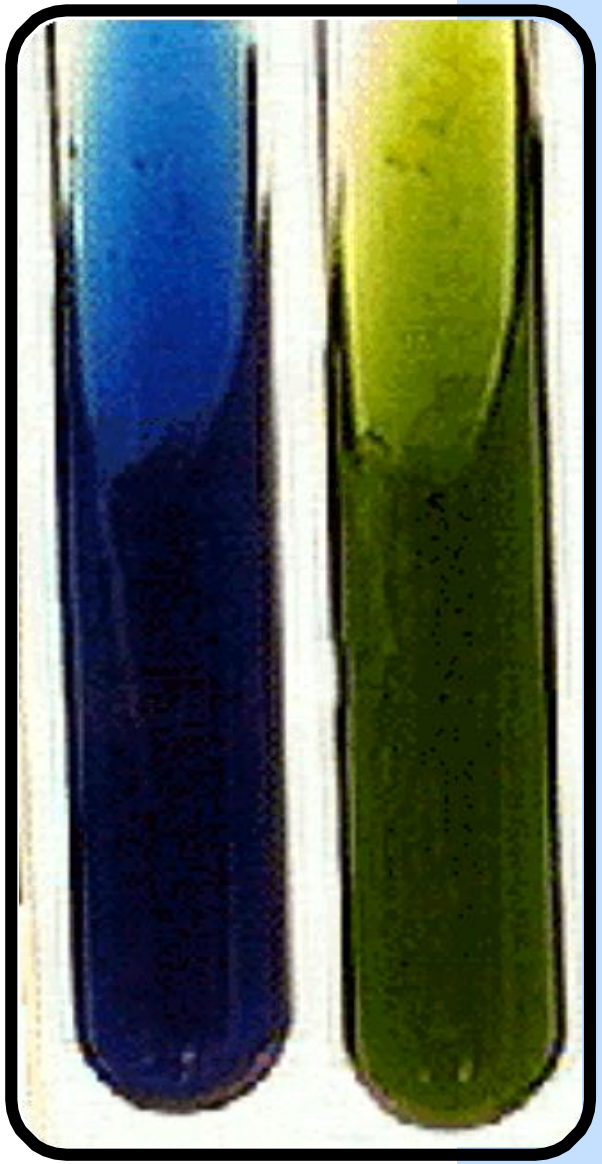
- **Indicator (color change) :**

Positive Test → High pH → Blue
Negative Test → Neutral pH → Green
(indicator used is **Bromothymol blue**)



4) Citrate utilization test

The indicator is bromothymol blue.



High pH

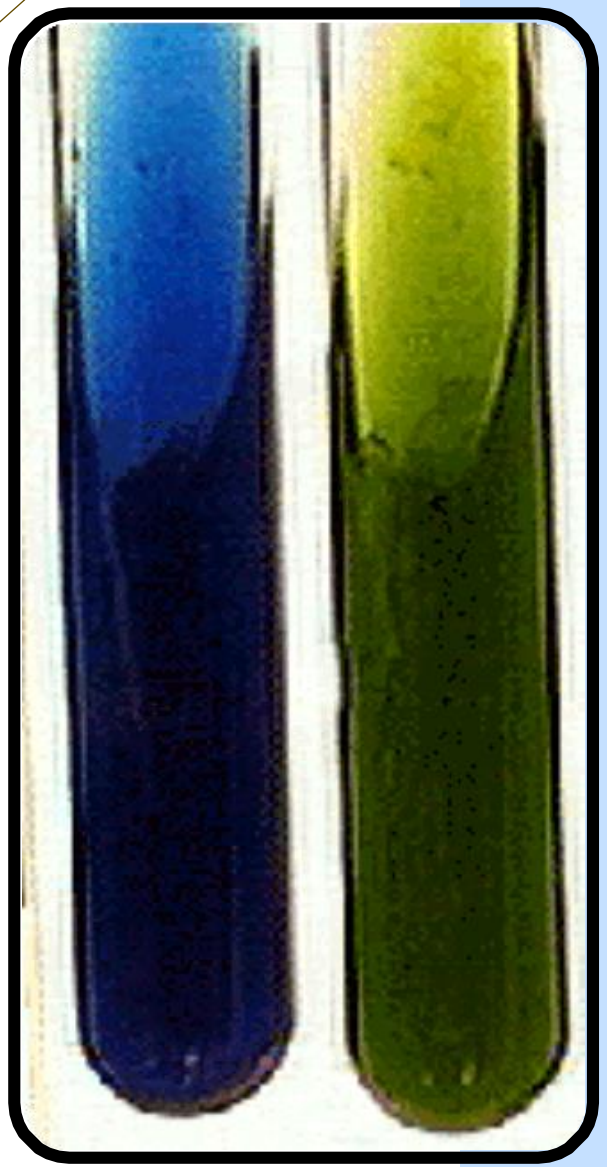
N

7

4) Citrate utilization test

Bacteria → Citrate medium

Only Citrate → As a carbon source



Incubate at 37°C for 48h

↓

Liberated Co_2

↓

Co_2+ with sodium

↓

Sodium carbonate (Alkaline)
Indicator change to blue

5) Urease test

- **Tests:** If the bacteria possess the urease enzyme.
- **How?** urea is added to the medium, If the bacteria possess the urease enzyme they will break down the urea. When bacteria break down urea by urease enzyme, ammonia (which is alkaline) is produced, which react with phenol red indicator.
- **indication (color change) :** phenol red is used as an indicator
  pink indicate a positive urease test
  yellow indicate a negative urease test

*If the medium turns yellow, it means the environment is acidic (it still in ureic acid form) indicating a negative urease test.

Phenol red indicator

Acid	Alkaline
6	8

5) Urease test

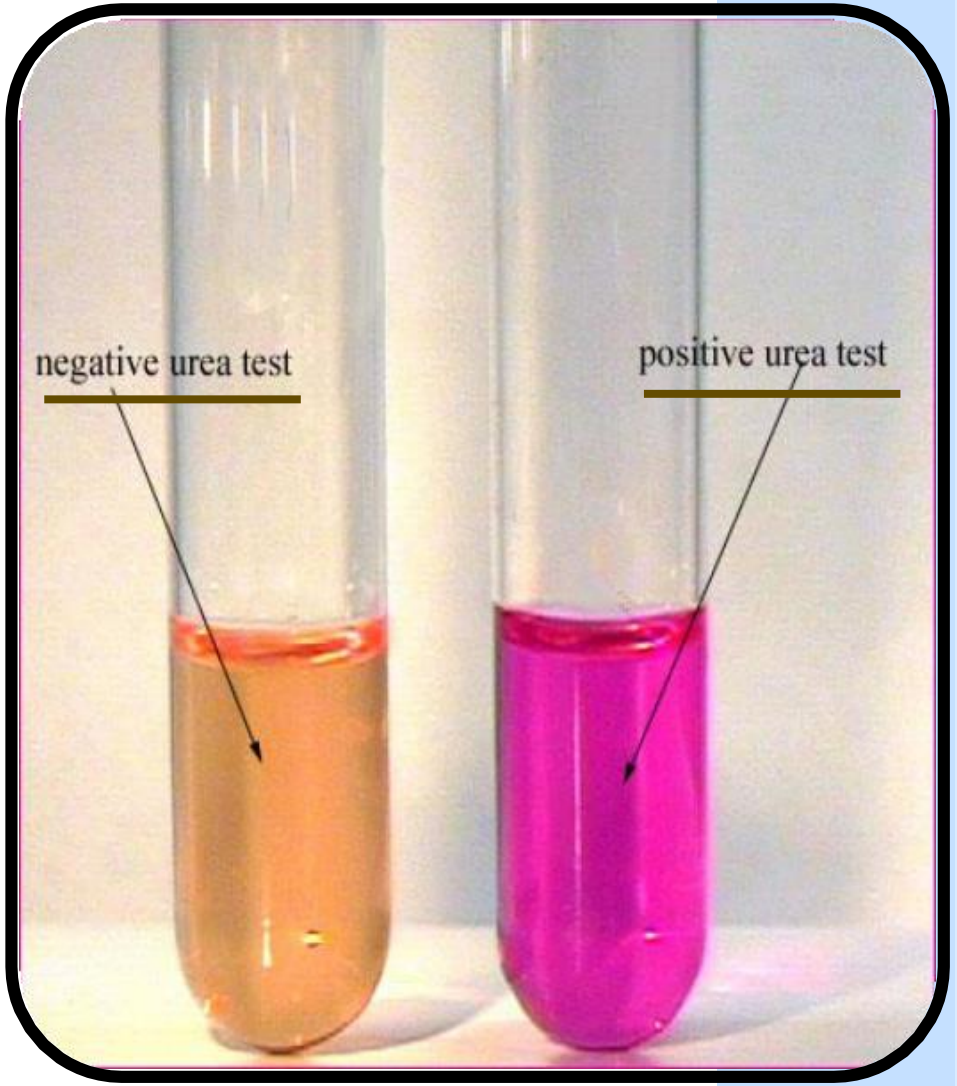
Adding it to the test tube

Bacteria (Urease)

Urea

Alkaline

Ammonia (Pink)



6) TSI (Triple Sugar Iron)

The medium used in this test is **semi-solid, with a gel-like gelatinous consistency.**

Medium Components:

0.1% glucose

1% lactose

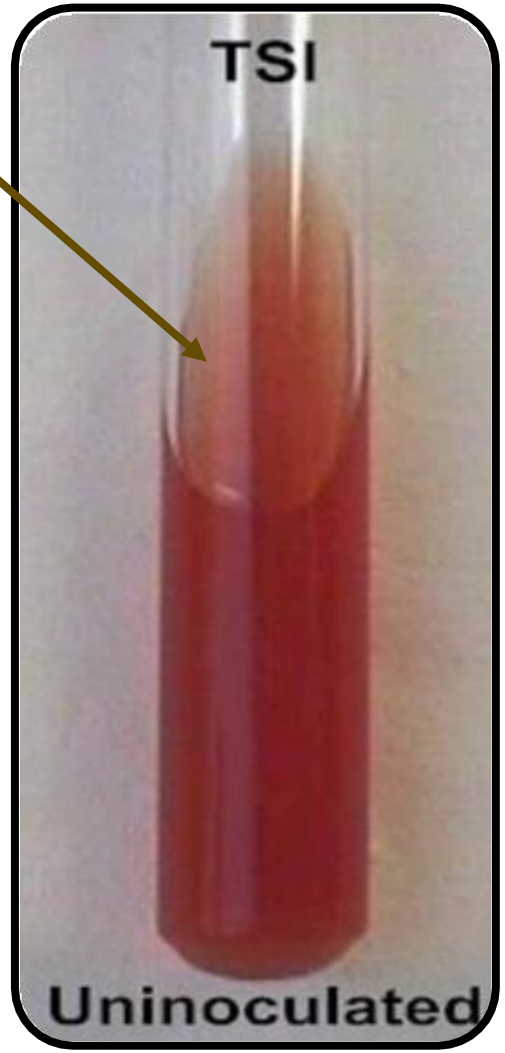
1% sucrose

Ferrous sulfate

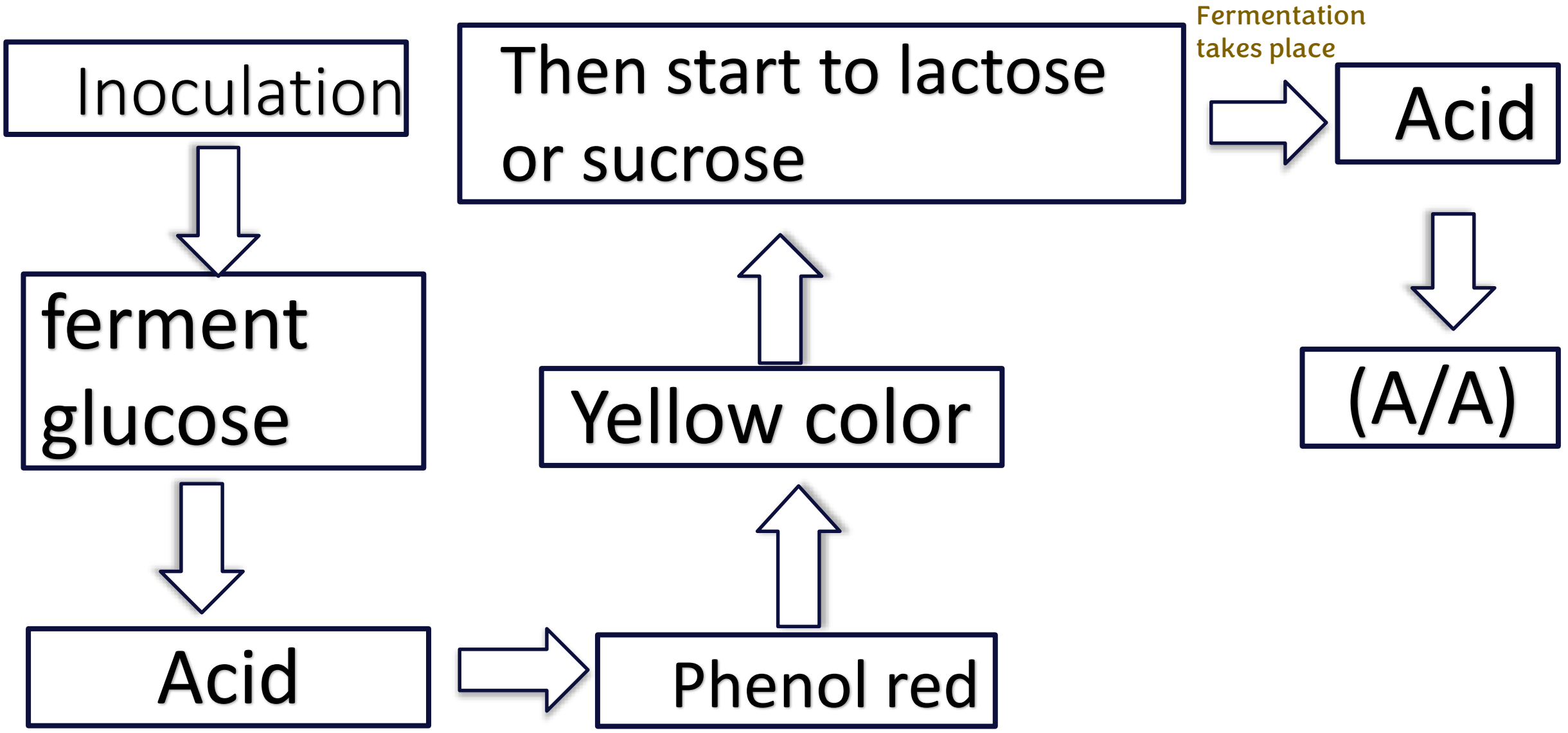
pH indicator: Phenol red

The test tube should be placed at an angle (obliquely) to have a slant on top.

When culturing the bacterial sample, we perform a stabbing motion at the bottom and streaking along the slanted surface.



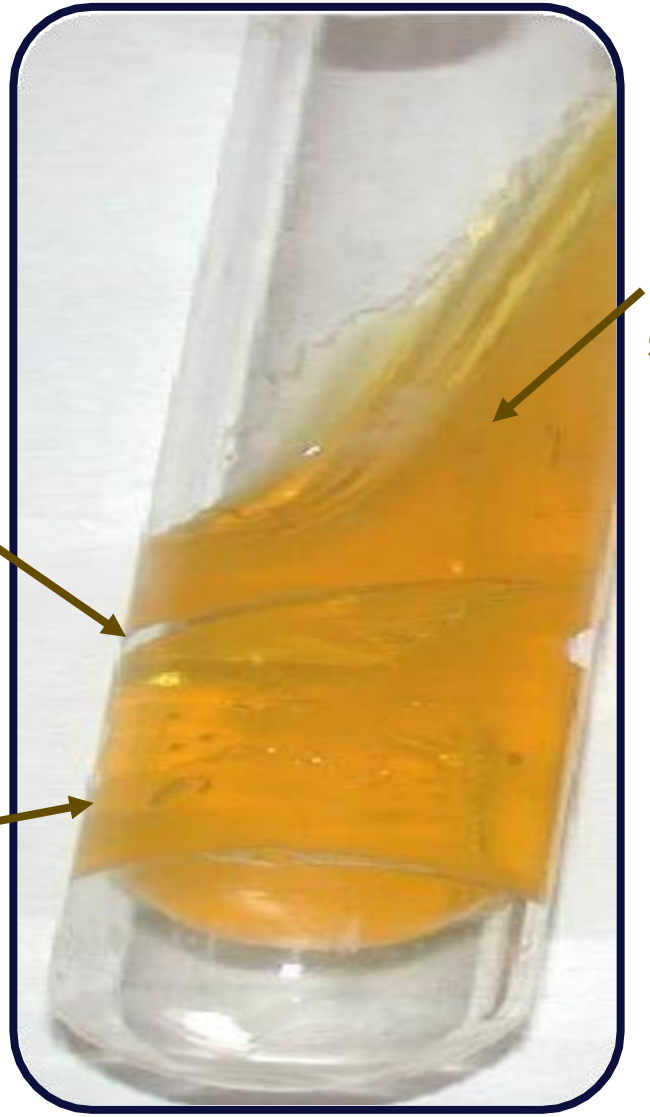
6) TSI a) Acid over acid (A/A)



6) TSI (a) Acid over acid (A/A)

If there is a break in the media and a slight elevation occurs, it indicates that the bacteria can produce gases.

Glucose is always at the bottom



Lactose and sucrose

a) Acid over acid (A/A)

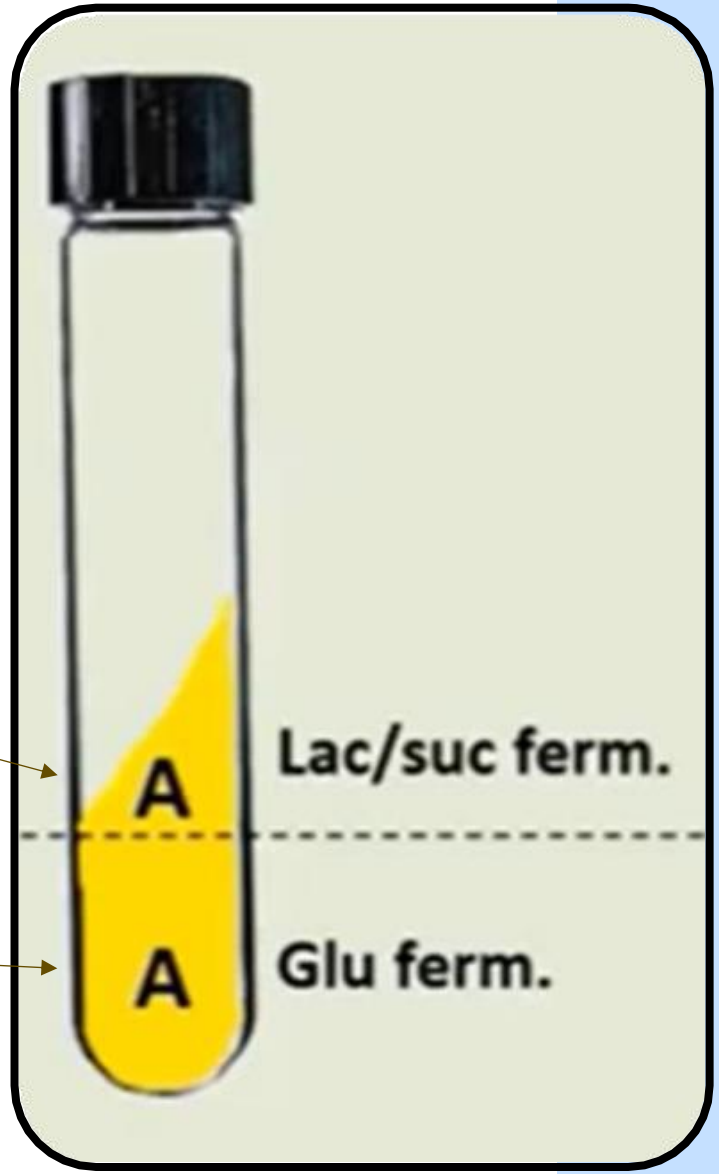
Detection of gas production by break up the medium or pushed up the tube.

a) Acid over acid (A/A)

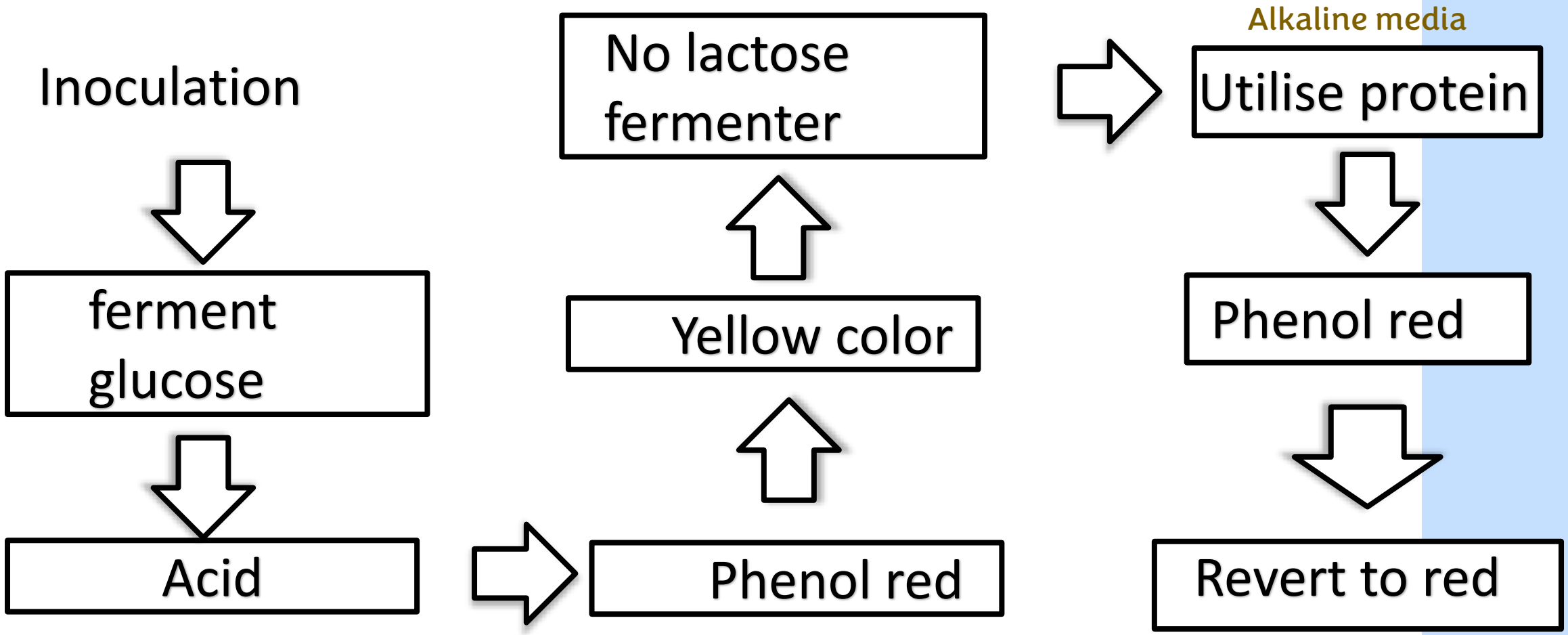
A/A

Lactose/Sucrose fermenter

Glucose fermenter



b) Alkaline over acid (K/A)



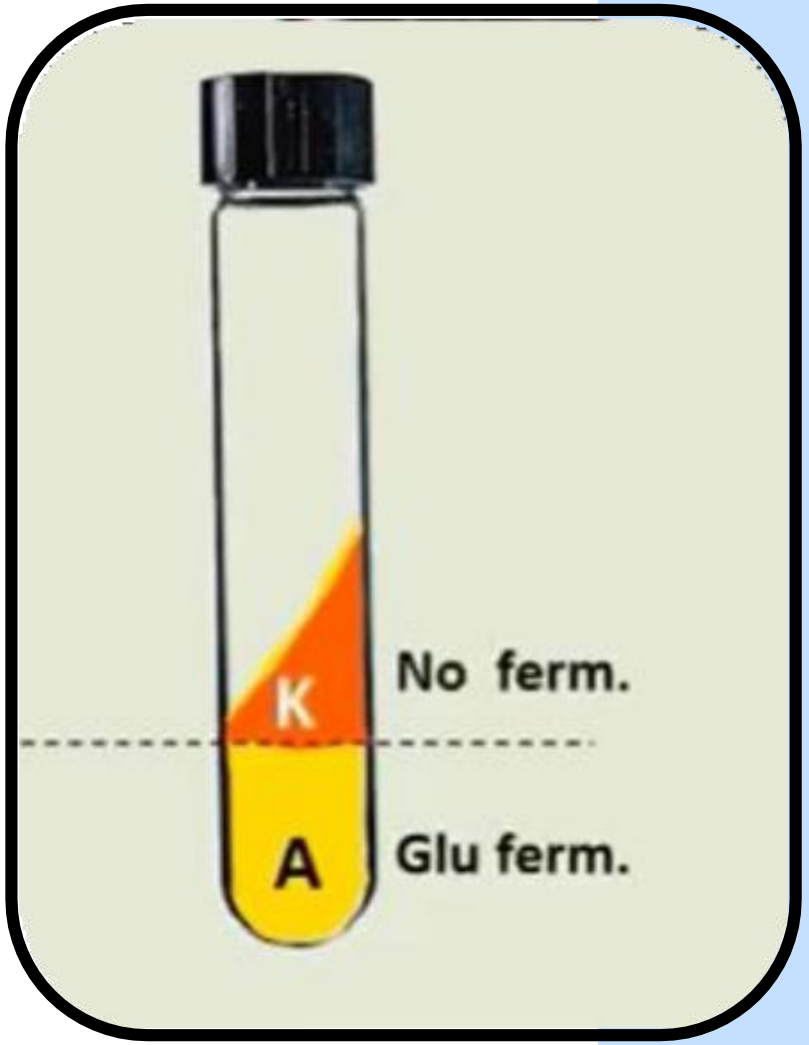
If the bacteria cannot ferment lactose, they will instead utilize the proteins present in the alkaline media. As a result, the media will remain alkaline, and the red color will persist, indicating no change. In contrast, the presence of a yellow color indicates successful fermentation of glucose.

a) Alkaline over acid (K/A)

K/A

Glucose fermenter

Non-lactose fermenter



a) Alkaline over Alkaline (K/K)

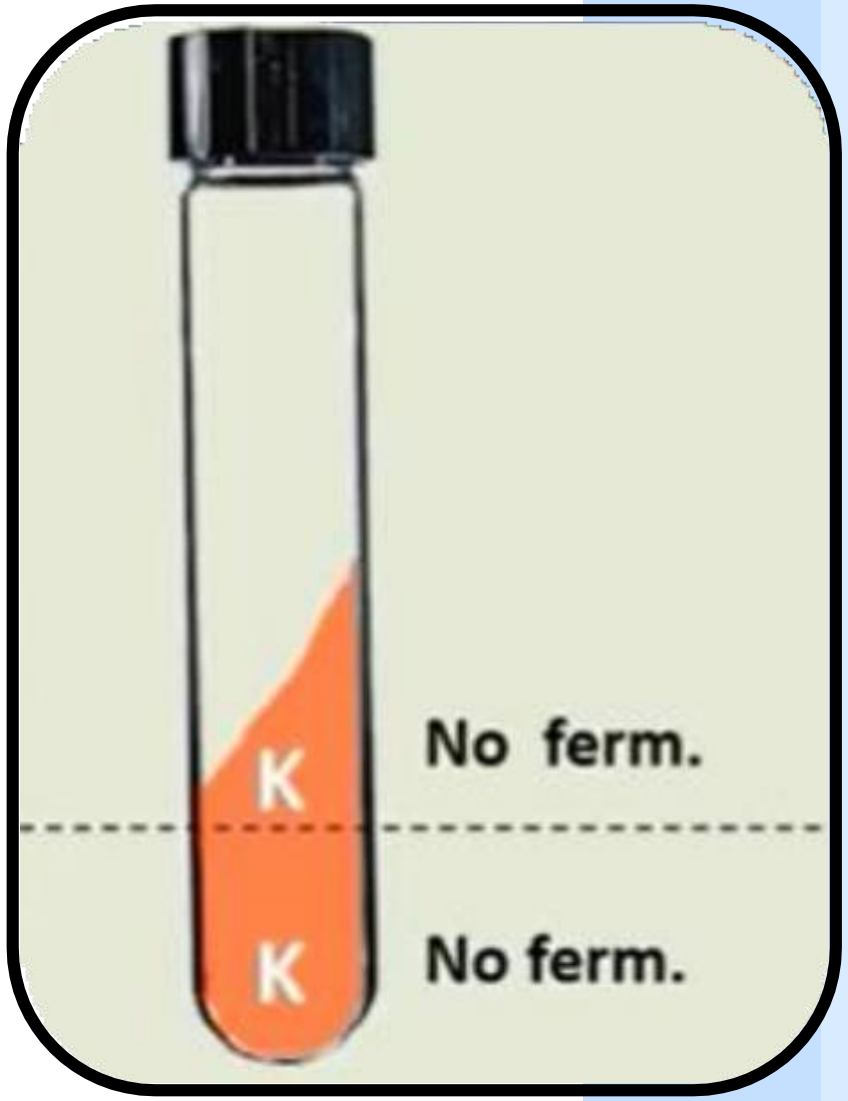
It is Neither a glucose fermenter
nor a lactose/sucrose fermenter

If the organism can not use the glucose in the medium. The color of the medium remains red.

a) Alkaline over Alkaline (K/K)

K/K

No sugar fermenter



H2S production

Bacteria (Reduce) → Sulfur

↓

Hydrogen sulfide(H_2S)

↓ iron

Ferric sulfide (Black)

Since the TSI test contains iron along with sugars, we can use the iron to determine whether the bacteria can produce hydrogen sulfide (H2S).

The presence of black color indicates the production of ferric sulfide (H2S) by the bacteria.

Summing up the TSI test:

A result of A/K is uncommon because usually if the organism can ferment lactose/sucrose, it can also ferment glucose

➢**Tests for (more than 1 thing):**

1. Whether the bacteria is a glucose fermenter
2. Whether the bacteria is a lactose/sucrose fermenter
3. Whether the bacteria can produce H_2S (use sulfur as e^- acceptor)

➢**How?**

1 & 2: by observing the medium change of pH due to fermentation

3: by observing the reaction between H_2S and iron (producing Fe_2S_3)

➢**Indication (color change):**

acidic alkaline

1 & 2: we have two compartments in the medium; yellow (A) → (+); red (K) → (-)

A/A → (+) for both 1 & 2; K/A → (+) for only 1; K/K → (-) for both.

3: if the reaction occurs, the black color of ferric sulfide is observed.

For 1 & 2, we use phenol red as an indicator. For 3, the black color is the color of ferric sulfide itself.

7) Phenylalanine deaminase

Phenylalanine deaminase

Enzyme

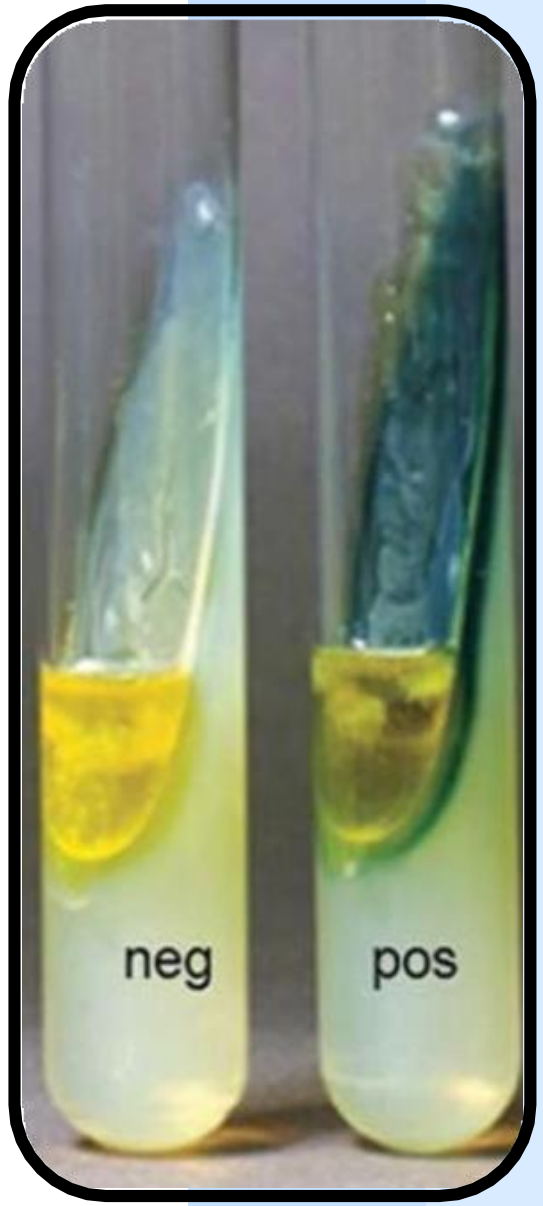
Phenylalanine → Phenyl pyruvic acid + NH_3

Substrate

Add Ferric chloride as indicator

Green color

Enterobacteriaceae is a family of bacteria that includes many members such as: *Proteus, Salmonella, and Shigella*.



- **Test is for**: determining whether the sample contains Salmonella or Shigella or not.
- **How?** as only these two ***don't*** contain Phenylalanine deaminase enzyme so green color would indicate the presence of proteus and not salmonella or shigella.
- **Indication:**
  no color change indicates (+ve Phenylalanine).
  green indicates (presence of phenyl pyruvic), so it would be Proteus.

Distinguishes *Proteus* from *Salmonella & Shigella*

8) Ornithine decarboxylase

Ornithine decarboxylase

⇩

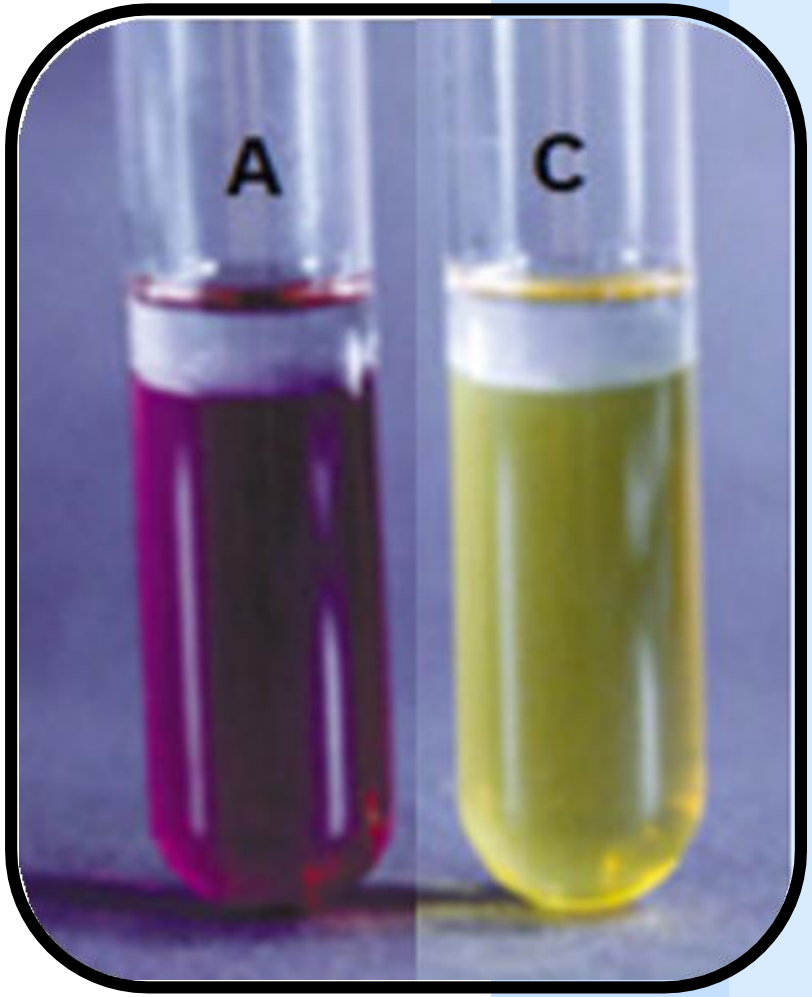
Ornithine

Source of carbon (energy for growth)

Providencia rettgeri (-ve) / Morganella morgani (+ve)

P. rettgeri & M. morganii

We use this test to distinguish between these two bacteria.



8) Ornithine decarboxylase

Since the tube contains not only ornithine but also glucose, the glucose is fermented first.

Organism

ferment glucose

Ornithine

Bromocresol purple (Indicator)

Yellow

Providencia rettgeri (-ve)

Purple

Morganella morgani (+ve)

Glucose

Ornithine

9) The analytical profile index (API)

- commercial stripe contains multiple tubes for various biochemical reactions.

- The analytical profile index (API)
- (Biochemical tests for identification)

We inoculate each tube, place them in an incubator, and after 24 hours, we record the color changes according to the catalog. This allows us to identify the type of bacteria.

9) The analytical profile index (API)

Color change

9) The analytical profile index (API)

- For example, if we have a Streptococcus bacteria and want to further identify which Species it belongs to, we use this type of test. Based on the color changes, we can determine which species we are examining.

Several API systems for different groups of organism.

Do not memorise

API 20E &API 20NE (Enterobacteria)

API 20 STREP (Streptococci) etc.

Oxidase test

Some bacteria produce Oxidase enzyme Detection by adding few drops of colorless Oxidase reagent Colonies turn deep purple in color (positive)

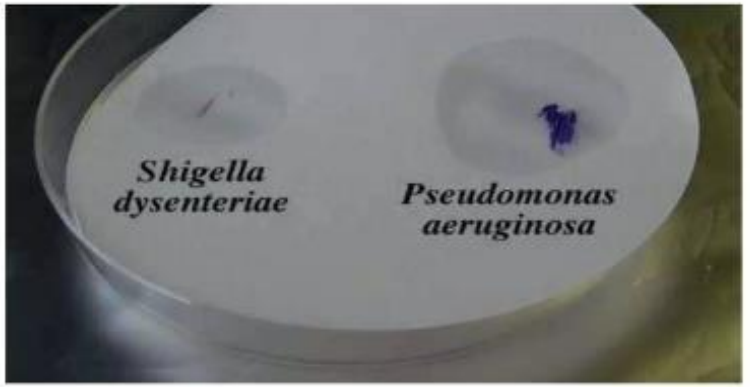
Oxidase Test

➢ **How?**

- All Enterobacteriaceae are oxidase-negative.

➢ **Test is used:**

- This test is used to differentiate enterobacteriaceae from *Pseudomonas* which is **oxidase positive.**



➢ **Indication (color change):** after adding colorless oxidase reagent
a purple color → indicates the presence of Pseudomonas bacteria.
no color is present →the bacteria is from the Enterobacteriaceae.

Catalase test

Some bacteria produce catalase enzyme.Addition of H2O2 lead to production of gas bubbles (O2 production)

We cannot rely only on morphology to differentiate between these bacteria; therefore, we should perform a catalase test.

- **Catalase test:**

➢ **Test:**

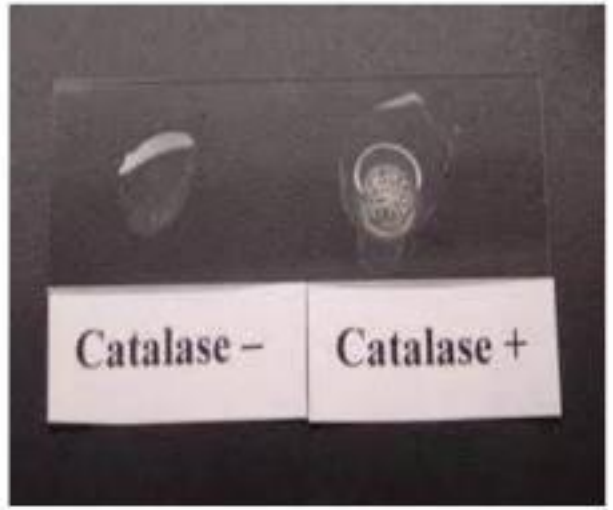
- Is used to differentiate between staphylococci(catalase +ve) and streptococci(catalase –ve).

➢ **How?**

- 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



Characteristic	Staphylococci	Streptococci
Catalase Test	Catalase positive	Catalase negative
Morphology	Forms clusters	Forms chains
Gram Staining	Gram-positive	Gram-positive
Shape	Cocci (spherical bacteria)	Cocci (spherical bacteria)

➢ **Indication:**

After adding hydrogen peroxide (H2O2), if bubbles are formed, it indicates that the bacteria are catalase-positive.

Coagulase test

- After confirming that it is Staphylococci, which are catalase-positive, we use a coagulase test to distinguish between the different members of this family.

Coagulase test:

Some bacteria produce coagulase enzyme Coagulase enzyme converts fibrinogen to fibrin (plasma clot)

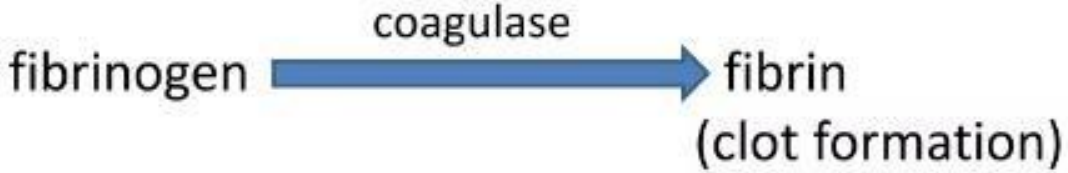
Detected by slide or test tube method

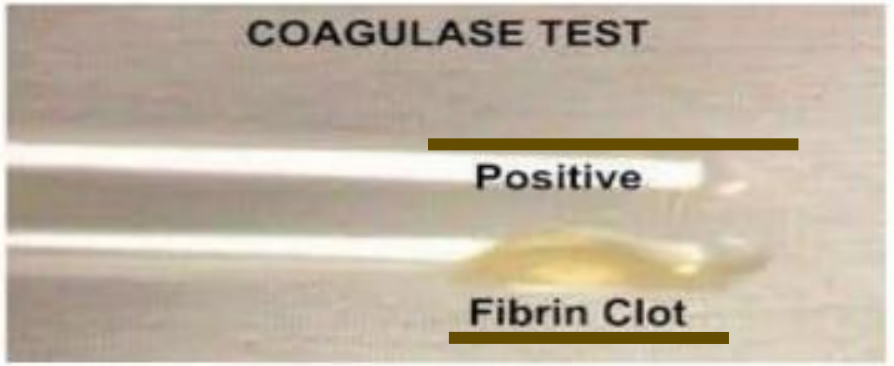
We can conclude that Staphylococcus aureus is:

1. gram-positive.
2. cocci-shaped, forms clusters.
3. catalase-positive, and coagulase-positive.

Coagulase test

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





- **How? (the principle) :** Staphylococcus aureus is the only member of the Staphylococcus family that is coagulase-positive.
- **Indication:** forming of fiber clot, indicate Coagulase-positive bacteria

Test	What it tests for ?	Principle (How it works)	Indication
1.Indole test	bacterium's possession of Tryptophanase	By adding tryptophane, that would give indol when broken which react with kovac's R	red color indicates a positive result.
2.Methyl red test	Whether bacteria can ferment glucose and produce a mixture of **acids**	By sensing the pH changes in the medium	red, indicating an MR-positive result. yellow, indicating an MR-negative result.
3.Voges-Proskauer test (V.P)	the ability of bacteria to ferment glucose, producing acetoin	Glucose→acetoine +alpha-alphanapthol +40%KOH→diacyetl (reacts with peptone)	red color, indicating a V.P-positive result.
4.Citrate utilization test	Presence of citrase (citrate lyase)	in a medium which has citrate as the only carbon source. If citrase is present, citrate will be metabolized, releasing CO_2 which combines with Na^+ forming Na_2CO_3.	Positive Test → High pH → Blue. Negative Test →Neutral pH→Green (indicator used is **Bromothymol blue**).
5.Urease test	If the bacteria possess the urease enzyme	If the bacteria break down urea by urease enzyme, ammonia (which is alkaline) is produced, which react with phenol red indicator	pink indicate a positive urease test yellow indicate a negative urease test.
6.TSI test	1. Whether the bacteria is a glucose fermenter 2. Whether the bacteria is a lactose/sucrose fermenter 3. Whether the bacteria can produce H_2S (use sulfur as e^- acceptor)	1 & 2: by observing the medium change of pH due to fermentation 3: by observing the reaction between H_2S and iron (producing Fe_2S_3)	1 & 2: we have two compartments in the medium; yellow (A) → (+); red (K) → (-) 3:the black color of ferric sulfide is observed.

Test	What it tests for ?	Principle (How it works)	Indication
7.Phenylalanine deaminase	determining whether the sample contains Salmonella or Shigella or not.	as only these two don't contain Phenylalanine deaminase enzyme so green color would indicate the presence of proteus and not salmonella or shigella	no color change indicates (+ve Phenylalanine) green indicates (presence of phenyl pyruvic), so it would be other Proteus.
8.Ornithine decarboxylase	to distinguish between *P. rettgeri* & *M. morganii*	tests for the presence of ornithine decarboxylase in M. morgani bacteria.	yellow, Providencia rettgeri (-ve). purple, Morganella morgani (+ve).
9.The analytical profile index (API)	A lot of tests combined	We inoculate each tube, place them in an incubator, and after 24 hours, we record the color changes according to the catalog. This allows us to identify the type of bacteria.	
10.oxidase	Used to differentiate Enterobacteriaceae from pseudomonas	All Enterobacteriaceae are oxidase- negative, except Pseudomonas which is oxidase-positive	a purple color → indicates the presence of Pseudomonas bacteria.
11.Catalase test	Used to differentiate between staphylococci and streptococci	Staphylococci is catalase-positive streptococci is catalase-negative	bubbles forming, indicates that the bacteria are catalase-positive.
12.Coagulase test	Used to differentiate staphylococcus aureus from other species under the staphylococci genus	Staphylococcus aureus is the only member of the Staphylococcus family that is coagulase-positive.	forming of fiber clot, indicate Coagulase-positive bacteria.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	42 43 53	**“(+) for none”** “How? as only these two contain Phenylalanine”	**“(-) for both.”** “How? as only these two *don’t* contain Phenylalanine “ the summary schedule changed according to slide 43
V1 → V2	43 53	**How?** as only these two *don’t* contain Phenylalanine deaminase enzyme so green color would indicate the presence of salmonella or shigella.	**How?** as only these two *don’t* contain Phenylalanine deaminase enzyme so green color would indicate the presence of proteus and not salmonella or shigella. Summary changed accordingly

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
(numbered in order as cited in the text)

1.Paper: DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. (DOI: 10.1099/ijs.0.64483-0)

الله أنجح ما طلبت به
والبر خير حقيبة الرجل.

اللهم استودعناك الأردن و شعب الأردن و قيادة الأردن و بلاد الشام و أهل بلاد الشام فاحفظهم عند عرشك المكين كما حفظت كتابك إلى يوم الدين
سبحانك الذي لا تضيع ودائعه