➢ Definitions / general information:

- Microbiology: Micro(small) – Bio(life) – Logy(science)

- Medical microbiology: is a science of studying micro-organisms (too small to be seen by naked eye) which associated with human disease, their activities and their influences on different aspects of life.

- Since organisms are widely distributed in nature, they can be beneficial or harmful. But medical microbiology deals ONLY with microbes that are harmful to humans.

***Beneficial:**

- **Food industry:** Fermentation of some products; Bread, Wine, Cheese, Yoghurt, Vinegar.
- **Industrial applications:** Bacteria is used in biotechnology such as genetic engineering, insulin, Enzymes, Amino acids, Vitamins, Antibiotics, Vaccines, Pharmaceutical industries.
- **Sewage treatment:** recycling water.
- **Recycling vital elements:** as nitrogen, carbon, oxygen, sulfur, phosphorus.

***Harmful:**

- **Food spoilage, Diseases.**

Microorganisms that cause disease are: pathogenic.
*Portal of entry:

- Respiratory: via inhalation.
- Alimentary (GIT): via ingestion.
- Genital tract: sexual contact.
- Skin: abrasions, bites.
- Congenital infections (vertical transmission) – *from mother to child through placenta & breastfeeding.*
- Others: conjunctiva / blood transfusion / injections / organ transplants.

➢ Scientists:

- **Antony van Leeuwenhoek:** (father of microbiology), a microscopist who was the first to observe live microorganisms in water mud and saliva.

- **John Hunter:** he was considered the leading authority on venereal diseases, and believed that Syphilis and Gonorrhea were caused by a single pathogen.

- **Edward Jenner:** A physician and scientist who pioneered the concept of vaccines including creating the smallpox vaccine, the world's first vaccine.

- **John Snow:** A physician, known for locating source of cholera outbreak in London (thus establishing the disease as water-borne), also he is considered one of the founders of modern epidemiology.

- **Ignaz Semmelweis:** A physician and scientist, known as early pioneer of antiseptic procedures. Described as the "savior of mothers", he discovered that the incidence of Puerperal sepsis can be prevented if the attending nurses apply hygienic measures. - Hand washing stops infections.

- **Louis Pasteur:** a biologist, microbiologist and chemist.
  - Discovered the principle of Fermentation of alcohol by microorganisms.
  - Invented a technique of treating milk & wine to stop bacterial contamination. - pasteurization process.
  - Created the first Vaccines of rabies, Bacillus anthrax.

  *** Louis Pasteur and the germ theory:**

  Performed numerous experiments → to discover why wine and dairy products became sour? → found that bacteria is the cause. → Pasteur called attention to the importance of microorganisms in everyday life & stirred scientists to think that if bacteria could make the wine "sick", then perhaps they could cause human illness! → However, Pasteur's attempts to prove the germ theory were unsuccessful.

- **Robert Koch:** provided the proof to germ theory, *how?* by cultivating anthrax bacteria apart from any other type of organism. Also, Developed microbiological media & streak plates for pure culture.

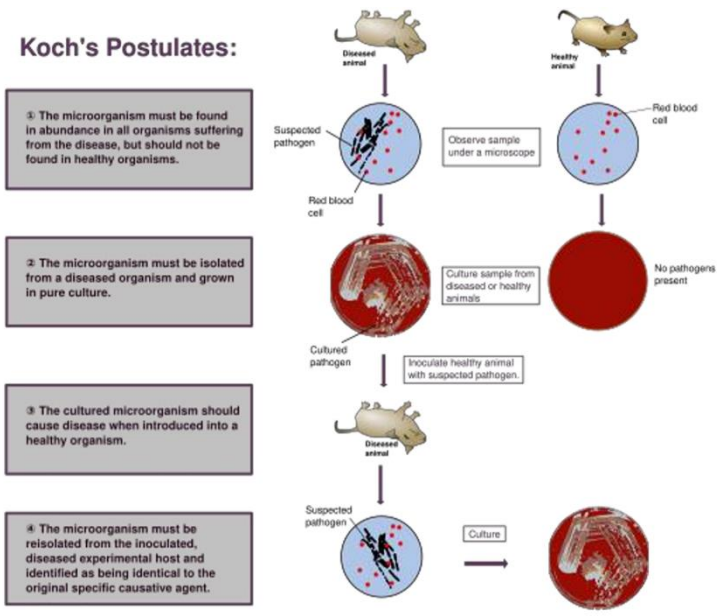
  ***Germ theory (Koch's postulates):**

  • Microorganism must be present in every case of the disease.

  • Organism must be grown in pure culture from the diseased host.

  • Inoculation of above into host must give same disease.

  • Organism must be recovered from experimentally infected host.



- **Alexander Fleming:** A physician and microbiologist, his best-known discovery the world's first broadly effective antibiotic (Penicillin G) from the mould Penicillium Rubens in 1928.

- **Kary Mullis:** A biochemist, invented Polymerase Chin Reaction (PCR) technique.

- **Zur Hausen:** A virologist, He has done research on cancer of the cervix, where he discovered the role of Papilloma viruses, this research directly made possible the development of a vaccine HPV.

اللهمَّ آتِنا في الدنيا حسنةً وفي الآخرةِ حسنةً ، و قِنا عَذَابَ النارِ

➢ Classifications:

*** 4 Classes of organisms that can cause disease:**

- **Viruses**
- **Bacteria**
- **Fungi**: within two varieties:
  - a- **Yeasts,** are unicellular (one cell) organisms.
  - b- **Molds,** are large multicellular organisms.
- **Parasites**: within two classes:
  - a- **Protozoa**, these are unicellular organisms that vary in size, some are very small and can cause intercellular infection. Others are large and cause extracellular infection.
  - b- **Helminthes**, these are multicellular and can reach several meters in lengths.

***Classifications of microorganisms:**

Eukaryotic	***Prokaryotic***	***viruses***
True nucleus Ex: Fungai, protozoa, algae 	Primitive nucleus Single chromosome Suspended (Nucleoid) Ex: Bacteria 	Acellular One of the smallest infectious agents No cell structure Obligate Intracellular Directed host cell for replication

classification	**Eukaryotic**	**Prokaryotic**
Nucleus	YES	NO
Size	10-100µm	0.05-10µm
Nuclear membrane	Yes (Nucleus)	No (Nucleoid)
Membrane-bound organelles Mitochondria, Golgi apparatus, ER	Present	Absent
Chromosome	Multiple (linear)	One (circular)
Ribosome	80S (40S -60S)	70S (30S-50S)
Cell wall	Absent EXCEPT Fungi (Chitin)	Present EXCEPT Mycoplasma
Cell membrane	Has sterols	No sterols EXCEPT in mycoplasma
Division	Mitosis	Binary fission

- **Viroid:** single-stranded RNA (ssRNA), circular, without any protective protein coat.
  It Infect Plants only! *Why? Since humans contains nucleases that will degrade foreign ssRNA, however plants lack it.*
- **Prion:** It is a Protein without nucleic acid (Infectious). > *happened due to:* protein misfolding.
  Aggregation of Prion in CNS → Spongiform in the brain → Creutzfeldt-Jakob disease (CJD) seen in humans.
  → Bovine spongiform encephalopathy (BSE) / Mad cow disease seen in cattle.

***Classification according to size:**

Fungi (largest) > Bacteria > Virus > Viriods > Prion (smallest).

- **Immunology**: since (Fungi, Bacteria, Virus, Parasites) are foreign substances then there is an immune response.

➢ Intracytoplasmic structures: 1) Nucleoid 2) Ribosome 3) Inclusion granules 4) Cell membrane 5) Plasmid

1) Nucleoid: > Essential … *(point of distinguish between prokaryotes and eukaryotes > bacteria have no nuclear membrane so nucleoid exists in the cytoplasm)*

-Single chromosome

-Circular

- dsDNA … ds=Double-stranded

-1mm in length …

-supercoiled…

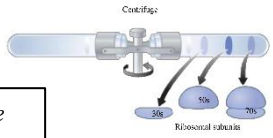
*Bacteria are typically within the size range of a few microns which is quite small, especially considering the amount of genetic material they need to pack inside. This is where **DNA supercoiling** comes into play, to compact their DNA so that it can fit within such a tiny space.*

-Carry genetic information for growth & survival.

2) Ribosome: > Essential

- Ribo=RNA, Some=body

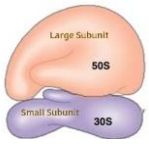
-Site of Protein synthesis > *recall: protein synthesis = mRNA + ribosome*



- Bacterial ribosomes > (70S) > (S) *refers to* Svedberg unit *measures* *density of both units.*

small & large subunits bind together when they bind with mRNA → The Small subunit's density is 30s, Large subunit's density is 50s → Their densities when together is 70… (not summative instead the total density using centrifugation) … So, bacterial ribosomes are indeed referred to as 70S ribosomes.

- Target of antibiotics 70S: 50S/30S *VS* Human 80S: 60S/40S



Very useful! Scientists used this difference in density to develop antibiotics that specifically attack bacterial ribosomes, without damaging our ribosomes.

3) Inclusion granules (bodies) > non-essential

- Function: Store of nutrient (Glycogen, Starch, Phosphate).

- Volutin granule (Metachromatic granules) >>> *These granules mainly store phosphate.*

4) Cell membrane > Essential

-Thin, fragile membrane located just inside the cell wall.

***Composition of cell membrane:**

- Phospholipid bilayer & Protein. Without sterols … *Except mycoplasma which have sterols.*

***Functions of the cell membrane:**

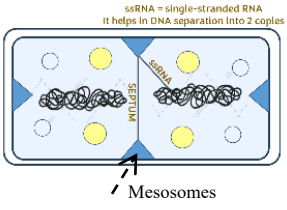
-1- Selective transport (Passive) / (Active).

Passive = Extracellular concentration is higher than intracellular concentration, so molecules get into the cell.

Active = Extracellular concentration is lower than intracellular concentration, so molecules need energy to get into the cell.

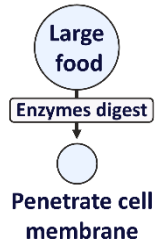
-2- Mesosomes are found as extensions of the cell membrane, where they contain enzymes linked to respiration and energy production. somewhat similar to the mitochondria in eukaryotic cells.

They play a role in cell division as well, by attaching to and assisting in the separation of DNA during replication into 2 copies. Specifically, septal mesosomes are thought to aid in forming the septum- the dividing wall that separates the two new cells during cytokinesis, by localizing at the center of the dividing cell, septal mesosomes help organize and distribute cellular components during division.

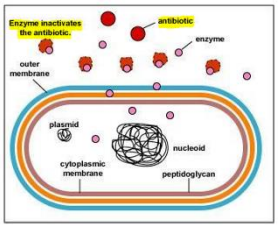


-3- Biosynthesis of cell wall… *synthesis the building blocks of cell wall.*

-4- Excretion of extracellular enzymes (Hydrolytic enzymes) & (Penicillinase).

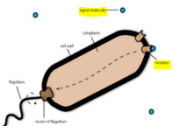


(Hydrolytic enzymes): microorganisms secrete these enzymes outside of their cells > enzymes break down complex macromolecules through hydrolysis > microorganisms can absorb these nutrients through penetrating cell membrane.



(Penicillinase): Some bacteria secrete enzymes (penicillinase) to defend themselves against antibiotics (penicillin). By doing this, bacteria prevent penicillin from inhibiting cell wall synthesis, allowing them to survive and continue growing > antibiotic resistance.

-5- Chemotactic system

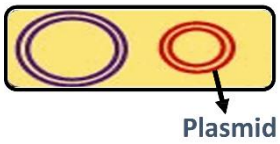


For bacteria that has flagella ONLY!

The cell membrane has receptors for certain materials. > When they detect a beneficial substance, they signal the flagella to propel the bacteria toward it. > Conversely, if a harmful substance is sensed, they signal the flagella to move the bacteria away.

5) Plasmid > Not essential

- EXTRA circular chromosomal dsDNA



**Why isn't plasmid considered as a part of the bacterial chromosome?*

- Replicate autonomously (Independent of bacterial chromosome).

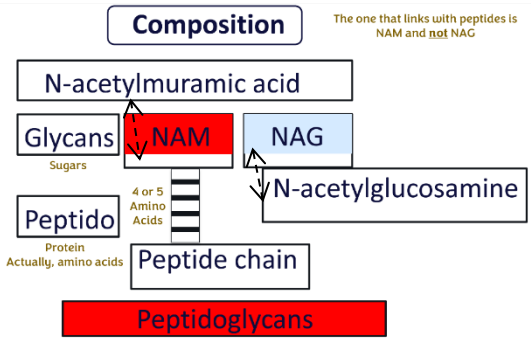
- Its genetic function is: Toxin production for Drug resistance … *whereas bacterial chromosome's function is survival & growth*

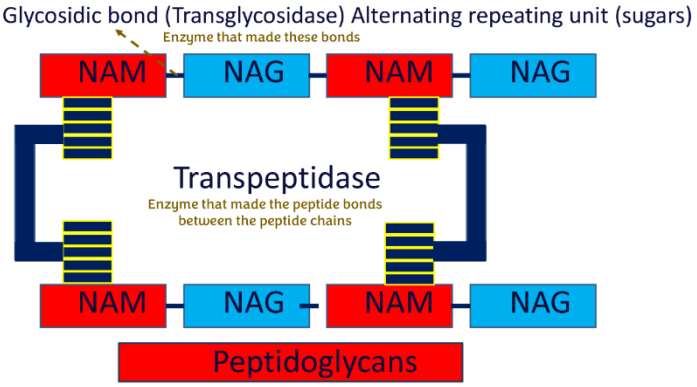
➢ Cell wall

- Outermost layer *(not always Tho, since capsule can be external to it)*, Surrounds the cell membrane.
- Rigid, its rigidity is due to Peptidoglycan.

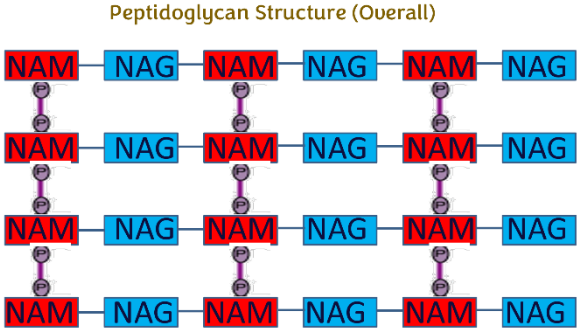
Composition of cell wall:** ***(very important!)

Zoom in:





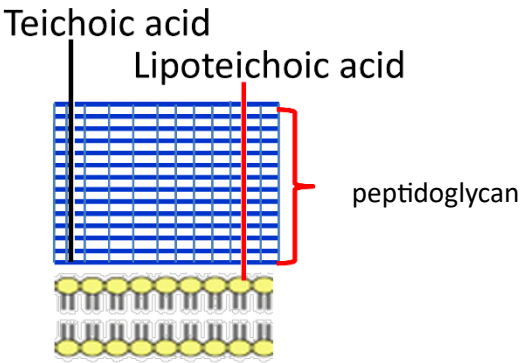
Zoom out:



***GRAM stain:** > *was created by Hans Gram.*

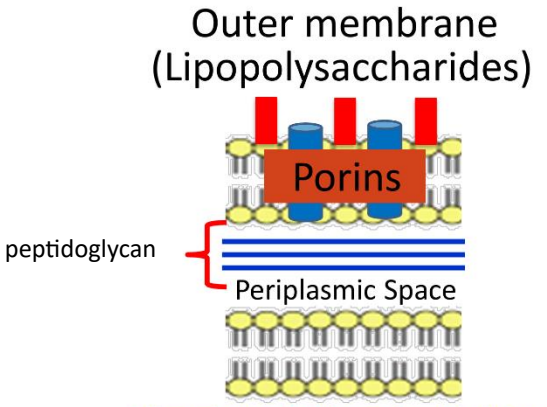
GRAM stain divides bacteria into two main groups (gram+ & gram -) each appears with specific color in the stain.

GRAM (+) *purple stained*



Cell membrane + THICK layer of peptidoglycans
Has teichoic acid (peptidoglycan-bound)
Has lipoteichoic acid (lipid-bound [to the cell membrane])

GRAM (-) *pink stained*



Cell membrane + THIN layer of peptidoglycans
Has an outer membrane

* *Composition of gram (+)*

1) *Peptidoglycan (50%)*

NAM-NAG

|

Peptide

(Porous)

2) *Lipoteichoic / Teichoic acid*

are composed of Polymers of Glycerol or Ribitol

- Lipoteichoic acid is (Cell membrane-bound)

- Teichoic acid is (Cell wall-bound)

Teichoic acids that are anchored to the lipid membrane are referred to as lipoteichoic acids (LTAs), whereas teichoic acids that are covalently bound to peptidoglycan are referred to as wall teichoic acids (WTA).

2) *Teichoic acid (specifically…)*

- Major surface Antigen of G (+ve)
- Highly immunogenic

Induces an immune response in our bodies because it is perceived as a foreign body.

Those are Cytokines Released after detecting the antigen

- { **TNF-α** } Tumor Necrosis Factor - Alpha
- { **IL-1**} Interleukin - 1

Teichoic acid is responsible for the Toxic Shock Syndrome (TSS)

* *Composition of gram (-)*

1) *Peptidoglycan A thin layer (5%)*

2 sheets of NAM - NAG

|

Peptides

2) *outer membrane*

A) Bilayer phospholipids

B) lipopolysaccharides

Lipid A (Endotoxin)
Toxin inside the GRAM (-)

Polysaccharides (somatic O Ag)
Antigen

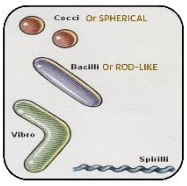
c) Porins (hydrophilic proteins) In the outer membrane (Transportation)

3) Periplasmic space

The space between the 2 membranes Space between cytoplasmic & outer membrane Consists of Peptidoglycan layer & gel-like protein

***Functions of cell wall:**

1. Maintenance of the shape (Rigid).



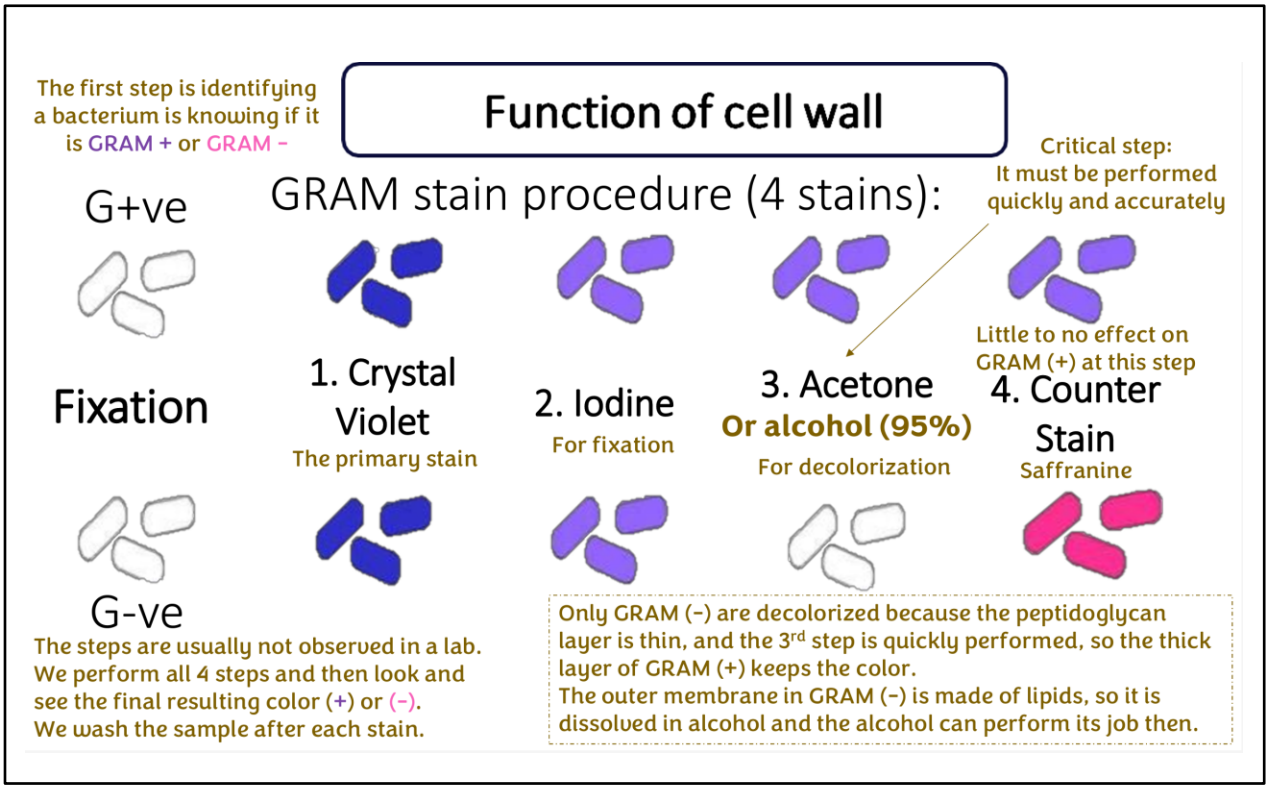
Different shapes depending on the cell wall!

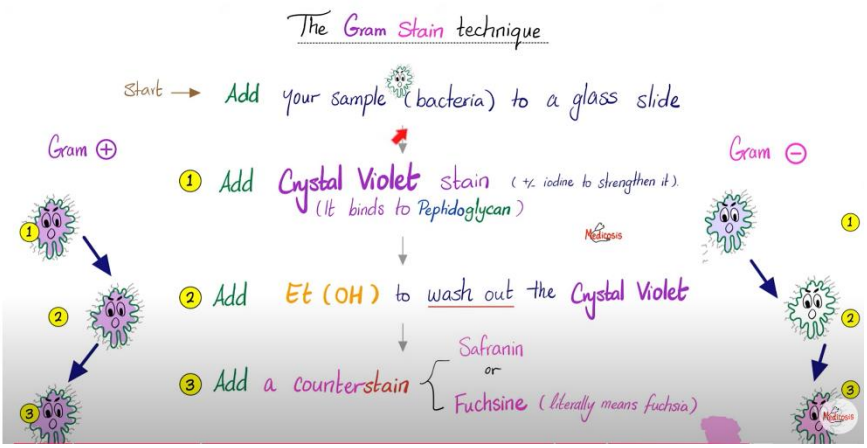
In case of:
- Deficient of cell wall > Polymorphic > Takes many shapes (no certain shape) > ex: MYCOPLASMA.

2. Protection (Osmosis insensitive) > Protects the cell membrane which is Osmosis Sensitive.

Recall the tonicity concept: If the cell is in a hypertonic solution → it shrinks. whereas, in a hypotonic solution → it lyses (bursts)… This effect is countered by the present of the cell wall.

3. Target site for antibiotics. Ex: Penicillin / Cephalosporines > *they attack the cell wall of bacteria.*
4. Role in cell division.
5. Responsible for staining.





**The gram stain is ruled by those bacteria with thick peptidoglycan cell wall (Gram +).*

**The thicker the wall the purpler the stain.*

Extra source: refer to this video for further explanation…
https://youtu.be/MI-FkBDamzw?si=2nrr14M31yFsOQ90&t=751

(رَبَّنَا اغفِر لي وَلِوالِدَيَّ وَلِلمُؤمِنينَ يَومَ يَقومُ الحِسابُ)

***Cell wall Deficient:**

- Bacteria without cell wall Cell membrane.

1) Naturally > ex: Mycoplasma (Sterol) > *Sterols (in the cell membrane) give some protection because this type lacks a cell wall.*

2) Induced > ex: 1- Cell wall inhibitors *(antibiotics)*, 2-Lysozyme *(from our body)*

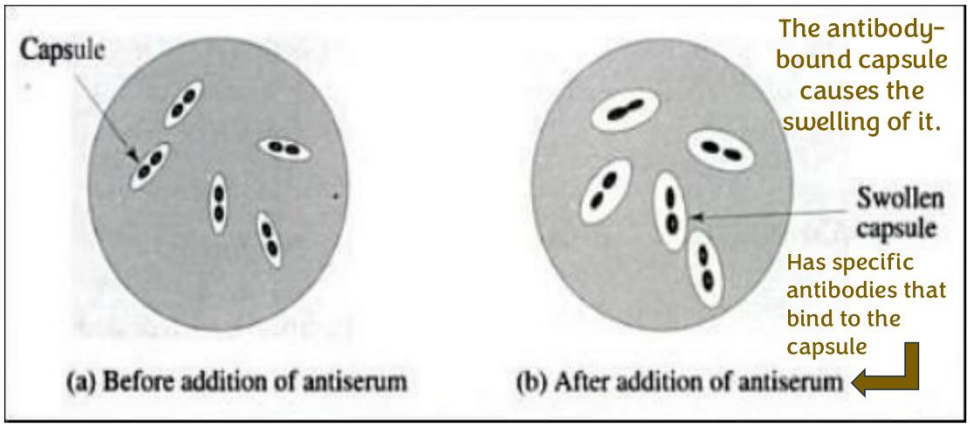
- Completely: Protoplast (G+ve) / Spheroplast (G-ve).
- Partially: L-form bacteria *(Named after Leister city in England :)*

Note: L-form bacteria & Mycoplasma resist Penicillin & Cephalosporines > *because their mechanism is attacking the cell wall*

➢ Structures outside the cell wall: 1) Capsule 2) Flagella 3) Pili 4) Spore formation

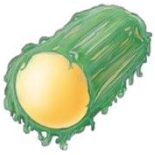
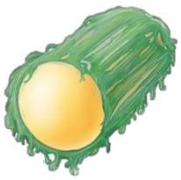
1) Capsule: > extra non-essential layer

- Glycocalyx: Glyco (carbohydrate) calyx (enveloped).
- A capsule is a wall of carbohydrates that surrounds the cell wall.
- It is a Gelatinous (Viscous) layer covering cell wall of some bacteria.
- Usually made up of > Polysaccharides. <u>Except (B. anthracis) > Polypeptides.</u>
- Variation of Capsule caused by different: (Arrangement of Polysaccharides). Ex: 91 types of Str. Pneumoniae.
- Do Not stain by Gram stain. We will often see unstained halo around the organism.
- Quellung reaction (swelling) > mechanism to identify if bacteria is capsuled.



** The serum contains **antibodies** that specifically bind to **antigens** present on the surface of the bacteria. Once the antibodies attach to the capsule, the capsule appears swollen and more visible under a microscope. This swelling is due to the interaction between the antibodies and the polysaccharides in the capsule.*

*** Capsule – composition**: > *name differs depending on the binding of the substance to the cell wall.*

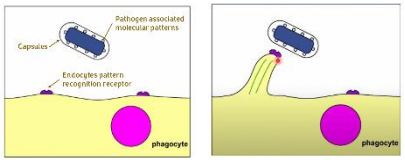
Capsule	Glycocalyx	Slime layer
- If the substance is highly attached to the cell wall, we call it a capsule -Tightly, organized bound around all cell wall - Firmly adherence to surface organism 	- Loosely & unorganized attached - Fibrils extending It, adhere firmly to skin & heart. - It adheres to the host cell NOT the bacterial capsule - e.g. Strept. Mutans 	- Loosely & unorganized attached 

***Function of capsules:**

1. Protect Cell wall; Bacteriophage - Complement system – lysozyme.

From Bacteriophage	*From Complements (in immunology)*	*From Lysozymes*
Infective virus that has specific receptors on the bacterial wall. When the cell wall is surrounded by capsules, it prevents bacteriophages from binding to the bacteria resulting in no infection.	(e.g: lectin & alternative pathway) The complement system must adhere to parts of the bacterial cell wall to start working. The capsule prevents the complement from binding > no infection.	Enzymes that degrade bacterial cell wall. > Presence of the capsule prevents the breakdown of the cell wall by lysozymes.

2. Prevent phagocytosis (Virulence).



It's a virulence factor (سلاح دفاع)

the capsule protects the cell wall from phagocytosis to protect itself.

(by "running away" from the phagocyte)

3. Capsules are formed in <u>VIVO</u> ONLY.

When the bacteria enter the host cell, they start forming the capsule by producing the components inside and secreting them to the outer surface of the bacterial cell wall.

*vivo vs vitro, VIVO: inside living organism. VITRO: outside living organism.

4. Attachment (Glycocalyx) > Dental caries & Prosthetic heart & valves.

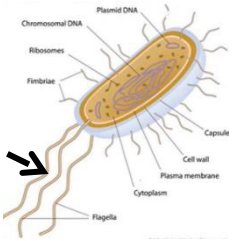
Dental caries	*Prosthetic heart & valves*
The sugars in the bacteria undergo fermentation and end up releasing acids that result in formation of dental caries. > The fibril extensions bind to any medical device (like implants, prosthetics.) in this case they adhere to the tooth enamel.	This is NOT a virulence factor since the glycocalyx high adherence (in this case to the heart) leads to diseases.

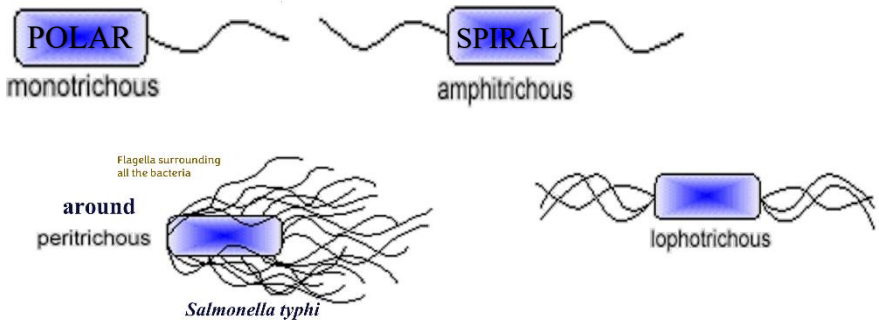
5. Development of vaccine.

This is performed by extracting the capsule of "Haemophilus Influenzae b" bacteria and binding it to a protein.

2) Flagella: > non-essential

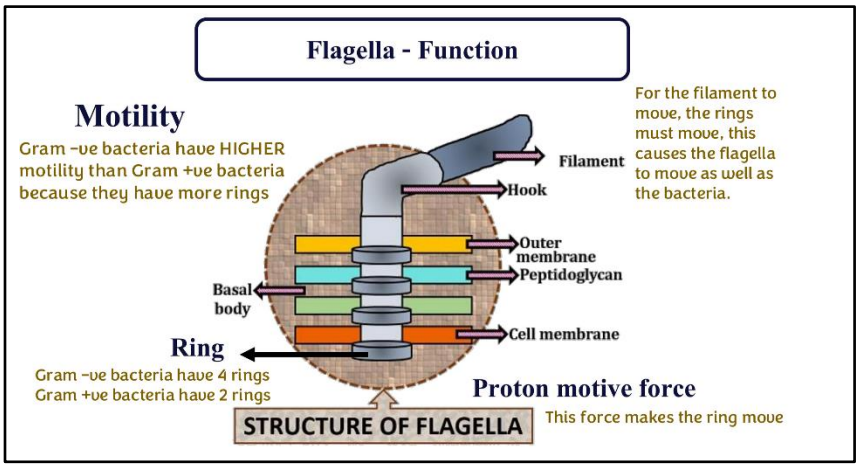
- Long thick threads like (filamentous) formed from protein (flagellin)
- Every flagella present in any bacteria is symbolized by (H Ag).
- Seen by EM (20nm) – small in size.





* Function of Flagella:

1. The organ of motility.



2. Tactic response (Taxis)

(Taxis) (Stimulus):

- response is due the cell's chemotactic system where cell membrane sends signals to direct flagella toward beneficial materials and away from harmful ones *(refer to cell membrane function – page 5)*
- (movement of bacteria toward (+ve chemotactic response) or away (-ve chemotactic response) from stimulating agent)

Types

Chemo Taxis > Stimulating agent is Chemical.
Photo Taxis > Stimulating agent is Light.

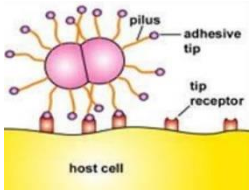
*Note: {Axial Filaments = Endoflagella} > internal flagella. Ex: spirochetes.

3) Pili (Fimbriae): > non-essential

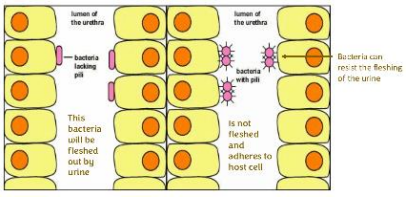
- Short and thin Hair like formed from protein (Pilin).
- Seen by EM.

*Types Of Pili:

a. **Ordinary pili (Attachment)** > virulence factor > resist fleshing of urine.

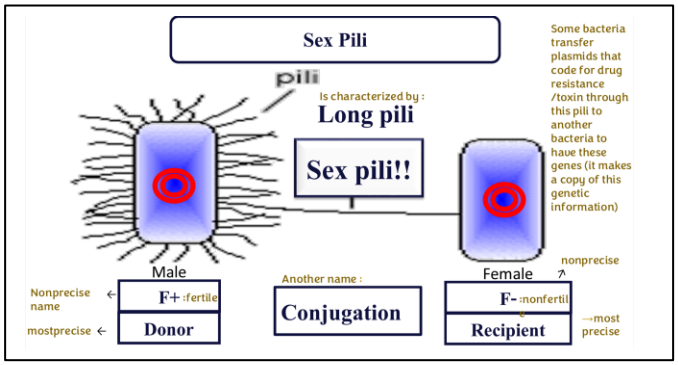


virulence factor: Because this pili acts as defending weapon and help in adhering of bacteria to the host cell in the first step of infection (establishing a disease).



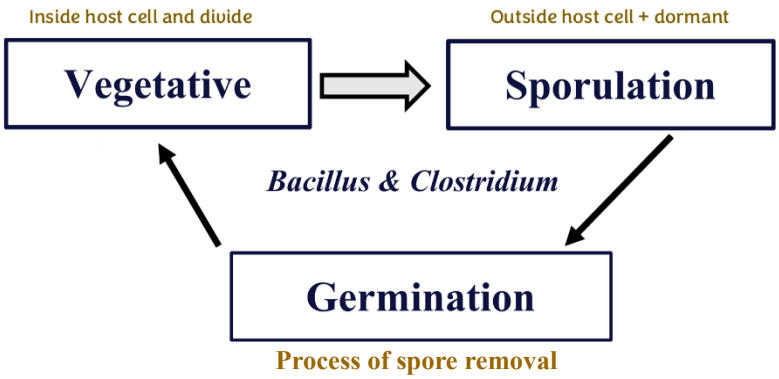
resist fleshing of urine: *(in urethra)*

b. **Sex pili (Genetic transfer)** = conjugation > long pili.



4) Spore formation: > non-essential

- **Vegetative bacteria → Unsuitable condition → Spore formation (Outside host cell).**
- Forming highly resistant resting phase (Endospores) in VITRO.
- Only 2 types can form spores: Bacillus & Clostridium.
- Occur to unfavorable conditions e.g. High temp, Drying, Depletion of nutrition.
- Formed outside the body (in VITRO).
- Can not stained by ordinary stain. *(specific stains are needed).*
- Spores are Highly resistant to dryness, heat & Disinfectant.



Spore formation concept:

Inside the host cell, some bacteria exist as **vegetative** cells, meaning they are in an active, growing, and dividing state, using the host's resources for nourishment. However, once they leave the host cell, they encounter **much less favorable conditions**. Outside the host, they may face:

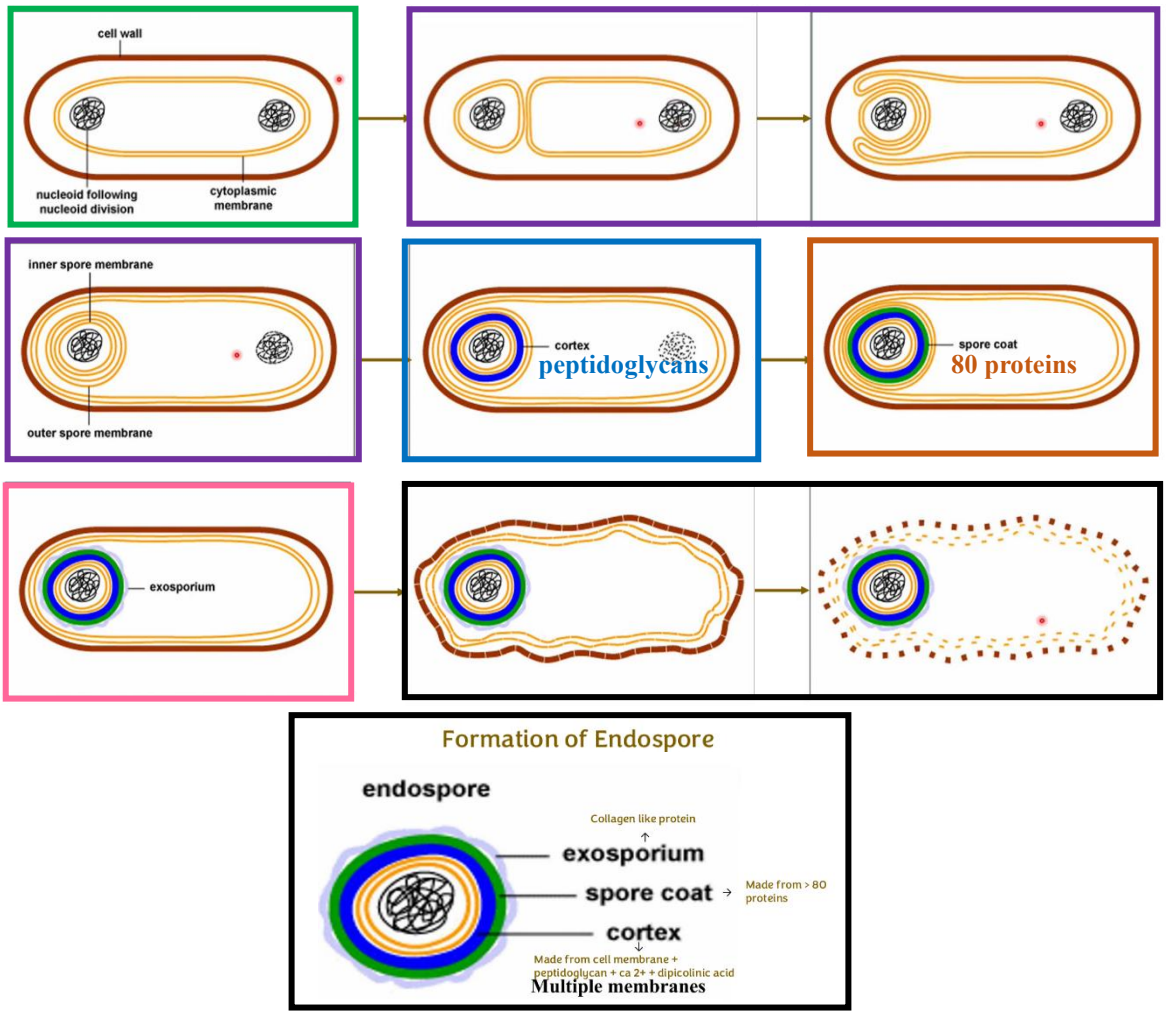
- High temperatures
- Lack of nutrients
- Exposure to disinfectants or chemicals
- Dehydration

These challenging conditions can be lethal to vegetative cells. To survive, certain bacteria {Bacillus & Clostridium} switch to **spore formation**. During sporulation, the bacteria transform from active, vegetative cells into **highly resistant resting phase** by forming **endospores**. Endospores are specialized, dormant forms that protect the bacterial cell's vital materials by surrounding them with thick, tough layers. Unlike regular, active bacterial cells (called vegetative cells), endospores do not carry out normal bacterial functions; instead, they stay in this "resting" state, waiting for better conditions.

When conditions improve, such as when the spore enters a new host or a more suitable environment, it can germinate back into a vegetative cell, resuming growth and replication.

*Doctor also mentioned that (gram +) are those who can form spores.

***Process of spore formation:**



1. **DNA Replication:** The process begins with the replication of DNA, where the bacterial DNA is copied. Each copy of the DNA then moves to opposite sides of the bacterial cell, preparing for the formation of two distinct cellular compartments.
2. **Formation of Protective Layers:** multiple layers of the cell membrane are formed. (inner/outer)
3. **Formation of cortex:** which is made up of peptidoglycans, this is formed between the 2 membranes. In which Calcium ions (Ca^{2+}) and dipicolinic acid (DPA) are synthesized and incorporated, which contribute to the toughness and heat resistance of the endospore.
4. **Spore Coat Formation:** Following the cortex, a spore coat develops. This coat is rich in proteins—over 80 different types that provide an additional protective barrier.
5. **Exosporium Formation:** An exosporium forms around the spore. This outermost layer is composed of collagen like glycoprotein, and further protects the endospore.

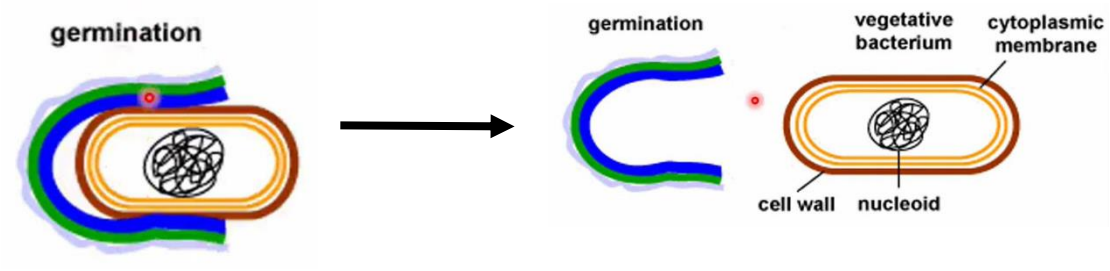
Once the spore is fully developed, the bacterium can exit the host cell and remain dormant, potentially surviving for centuries in harsh conditions.

Layers that form from inside to outside: Cortex → Spore Coat → Exosporium.

* Process of Germination:

Germination of spores occurs when the bacteria find suitable conditions like availability of water.

In germination, bacteria will break down all the layers (cortex, spore coat, exosporium) and return to become vegetative bacteria and begin their activities inside the host cell.

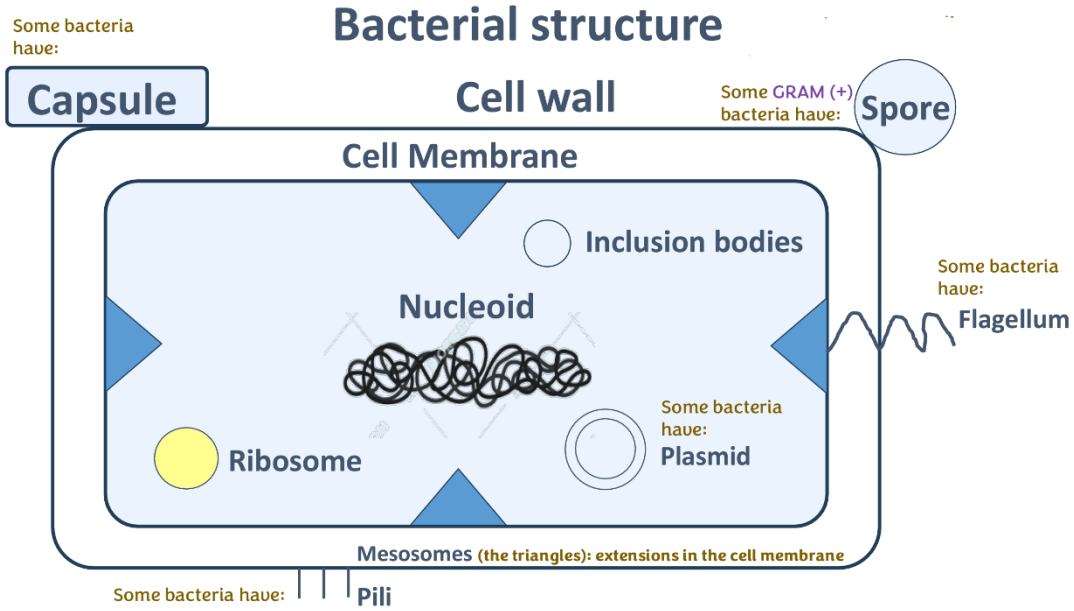


تذكير3> : من لطف الله و رحمته اقتصار ال spore formation على These two types of bacteria... فسبحان الله و الحمدلله ولا إله إلا الله و الله أكبر...

*Position of spores: > *Depends on the location of spore.*

Central & Oval	*Sub- terminal & Oval*	*Terminal & Spherical*
		
B. anthracis	***Cl. perfringens***	***Cl. tetani***

Recap: bacterial structure overview: -



➢ Bacterial taxonomy

- Taxon= group, Taxa= groups = classification. >> <u>The science of biological classification.</u>
- Taxonomy discuses 1-classification, 2-nomenclature & 3-identification.

1- *Bacterial taxonomy rank (classification):

- Kingdom or Domain
- Division or Phylum
- Class
- Order
- Family
- Genus - genera
- 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.

- situation (1):

Species:

A collection of strains that share many stable properties

Such as: Genetic and morphological properties.

→ ***Strains:***

Individual member within a species

* *Example:* Staphylococcus aureus is a species that consists of many strains, these are some.

Staph. aureus (Species) →

MRSA (Strain)

Methicillin-resistant Staphylococcus aureus

VRSA (Strain)

vancomycin-resistant staphylococcus aureus

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.

***IF AND ONLY IF: {(DNA homology ≥ 70 %) & (16 S rRNA > 97 % identical)}**

- situation (2):

Genus:

One or more species that share common properties.

→ ***Species***

Staphylococci (genus) →

S. aureus (species)

S. epidermidis (species)

Bacteria are classified within the same genus:

if they have at least 93% DNA homology (using ANI technique)

***IF AND ONLY IF: (DNA homology > 93 %)**

Q: how is the DNA homology limit higher for genera, although it's a wider group?

Ans: two different techniques were used. If we used ANI technique for the species criteria the limit would be >95% of homology

2- *Nomenclature:

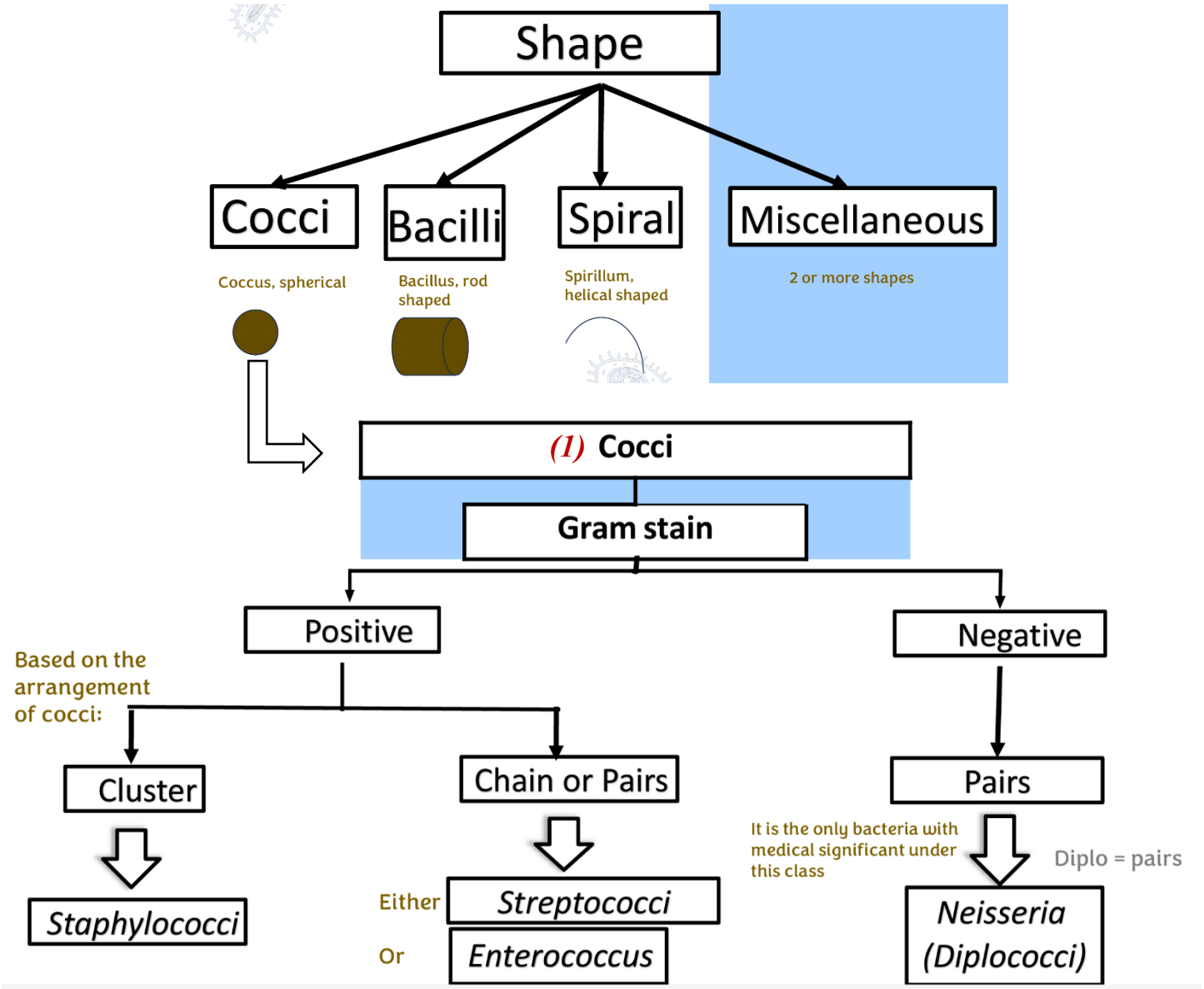
Example: *Escherichia coli*

1) A name must consist of 2 words, the first is the genus, the second is the species. **long form {Genus + species}**
2) First letter of genus name should always be capitalized.
3) The species should be written all with small letters.
4) The name should be written in italic or underlined (an old way).

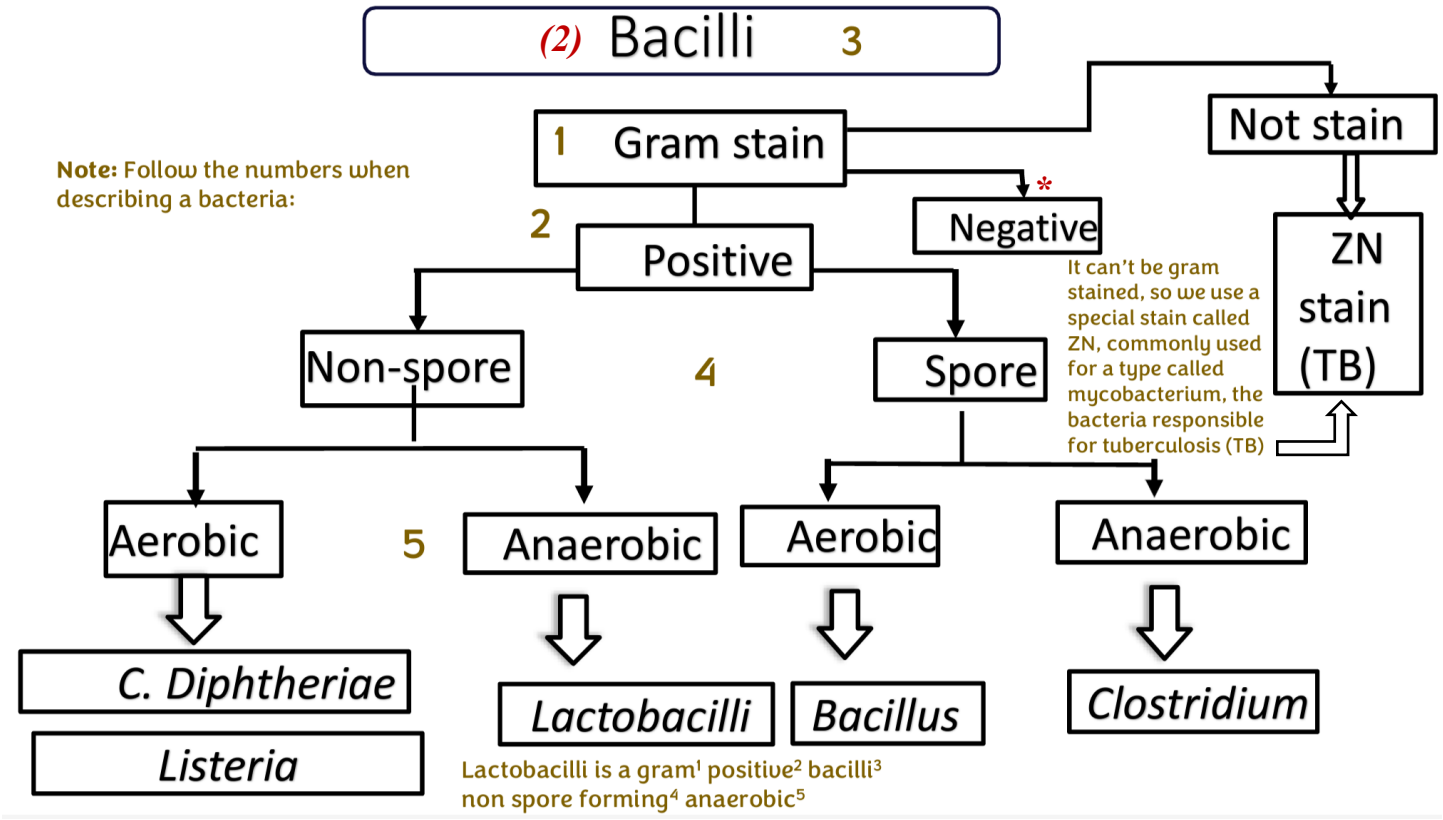
Example: *E. coli*

Can be written using **short cut form**: (FIRST LETTER OF GENUS NAME. species name).

3- *Scheme of medical bacteria (identifications):



اللَّهمَّ إنِّي ظلَمتُ نَفسي ظلمًا كثيرًا ولا يغفرُ الذُّنوبَ إلَّا أنتَ فاغفِر لي مغفرةً من عندِكَ وارحَمني إنَّكَ أنتَ الغفورُ الرَّحيمُ.



Gram negative bacilli

❑ *Enterobacteriaceae* ❑ *Vibrio* ❑ *Campylobacter* ❑ *Helicobacter* ❑ *Pseudomonas*

❑ *Haemophilus* ❑ *Bordetella* ❑ *Brucella* ❑ *Legionella* ❑ *Gram -ve anaerobes*

(3) - Spiral

❑ *Treponema* ❑ *Borrelia* ❑ *Leptospira*

(4) - Miscellaneous group

❑ No cell wall

❑ Not stain by gram

❑ Obligate intracellular

Names: ❑ Mycoplasma ❑ Chlamydia ❑ Rickettsia ❑ Coxiella ❑ Actinomycete

- **Systematic Bacteriology** (bacteria related to human body systems) is often discussed based on the following criteria:

  1- Morphology & Culture 2- Virulence factor & Pathogenesis 3- Diseases 4- Lab Diagnosis 5-Treatment & Prevention.

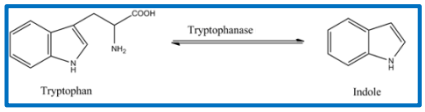
➢ Biochemical reactions

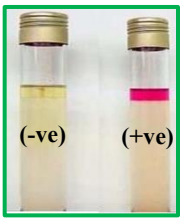
Some extra resources:

1- https://youtu.be/IlRU_NNYGe8?si=PHIk5n1kCWKCRzOC
2- https://youtu.be/E4a8g1o72AM?si=ZLCjYlycMwBatAnO
3- Summary – modified slides L4 – page 52 (يعطيهم العافية 3>)

1) Indole test

bacteria → **Peptone (TRYPTOPHAN)** → **Indole** + **Kovac' s R.** → **red**





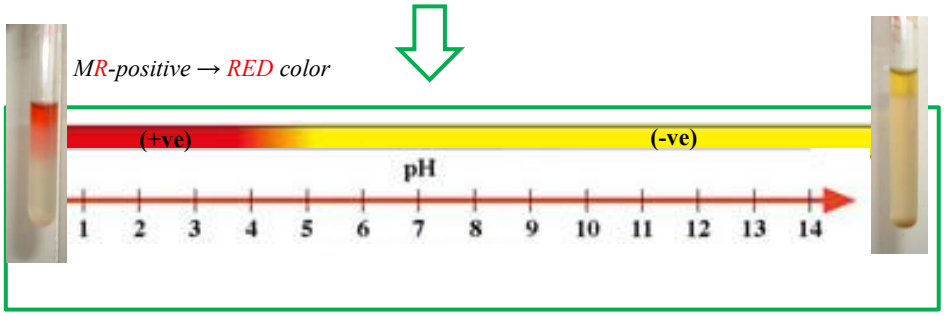
Tests for: bacterium's possession of Tryptophanase.

How? breaking down tryptophan by Tryptophanase in the medium and produce indole which reacts with Kovac's reagent.

Indication (color change): a red color indicates a positive result.

2) Methyl red test

Organism → **Glucose** → *Incubate at 37 ° C for 48h* → **Large amount of Mixed acid (Acetic, lactic, & succinic)** → *MR-positive → RED color*



Tests for: Whether bacteria can ferment glucose & produce a mixture of acids.

How? 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.

Acid produced → PH < 4 → red

Acid isn't enough → PH > 6 → yellow

Further explanation: 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.

3) VogesProskauer test (V.P)

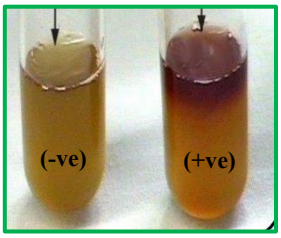
Organism → **Glucose**

Incubate at 37 ° C for 48h

Acetyl methyl carbinol (Acetoin)

alpha-naphthol + **40% KOH**

Diacetyl → **red**



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

How? 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. if acetoin is present, it gets oxidized to diacetyl, which reacts with peptone in the medium.

Indication (color change):

a red color, → V.P. positive result.

! ***If MR is +ve, then VP should be -ve.** If the bacteria ferment glucose and produce acid, They typically, do not produce acetoin, and vice versa.*

4) Citrate utilization test

Bacteria → **Citrate medium**

Incubate at 37 ° C for 48h

Liberated CO2

CO2 + **sodium**

Sodium carbonate (Alkaline) → **blue**

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.

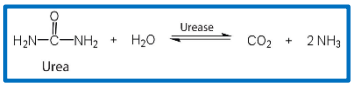
How? By leaving the bacteria in a medium that has citrate as the only carbon source. If citrase is present, citrate will be metabolized, releasing CO2 which combines with Na+ forming Na2CO3 (sodium carbonate) which increases pH of the medium. If no citrase is present, nothing happens, and the pH stays close to neutral.

Indication (color change):
Positive Test → High pH → Blue
Negative Test → Neutral pH → Green
(indicator used is Bromothymol blue)

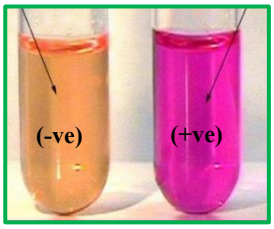
Note: bromothymol blue in normal cases is green. If alkaline (high PH, usually more than 7) is detected. bromothymol is converted to blue.

5) Urease test

Bacteria → **Urea** → **Ammonia** → **pink**



Note: phenol red is yellow/orange in acidic solutions. While red/pink in basic solutions. So, if the medium turns yellow, it means the environment is acidic (it still in ureic acid form) indicating a negative urease test.



Tests for: If the bacteria possess the urease enzyme

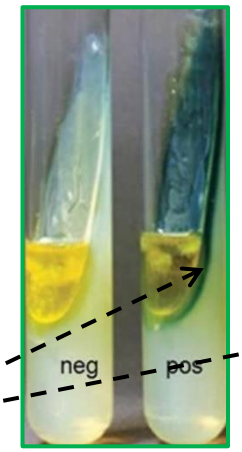
How? urea is added to the medium, If the bacteria possess the urease enzyme they will break down the urea. When bacteria break down urea by urease enzyme, ammonia (which is alkaline) is produced, which react with phenol red indicator.

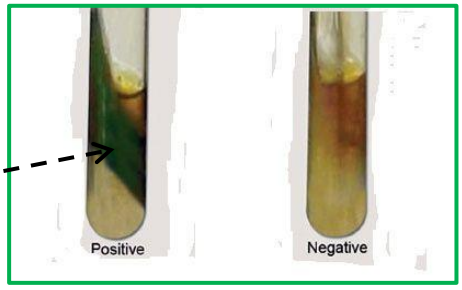
Indication (color change):

pink indicate a positive urease test
yellow indicate a negative urease test (phenol red is used as an indicator)

6) Phenylalanine deaminase

Bacteria → **Phenylalanine** → *Phenylalanine deaminase* → **Phenyl pyruvic acid + NH3** → **Green**





Tests for: Determining whether the sample contains Salmonella / Shigella or not. → Distinguish Proteus from Salmonella & Shigella

How? Phenylalanine deaminase breaks down phenylalanine into phenyl pyruvic acid and ammonia (NH_3). Adding ferric chloride as an indicator causes a reaction with phenyl pyruvic acid, producing a green color if the acid is present. This helps distinguish bacteria: since only Proteus species contain the phenylalanine deaminase enzyme, while Salmonella and Shigella lack it. So, a green color indicates the presence of Proteus, whereas no color change suggests Salmonella or Shigella.

Indication (color change):

no color change → (+ve Phenylalanine).

green → (presence of phenyl pyruvic) → (-ve Phenylalanine) → Proteus.

(Ferric chloride as indictor)

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

7) TSI (Triple Sugar Iron)

- Situation (a): **A/A**
- Situation (b): **K/A**
- Situation (c): **K/K**
- Situation (d): **H2S production**

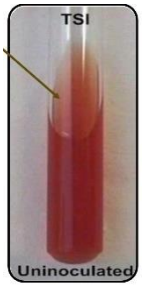
A/K is uncommon because usually if the organism can ferment lactose/sucrose, it can also ferment glucose

***In general:**

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

it contains:

- 0.1% glucose
- 1% lactose
- 1% sucrose
- Ferrous sulfate (Fe2S3)
- 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.

Tests for:

1. whether the bacteria is a glucose fermenter.
2. Whether the bacteria is a lactose/sucrose fermenter.
3. Whether the bacteria can produce H2S (use sulfur as e- acceptor)

How?

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

3: by observing the reaction between H2S and iron → producing Fe2S3.

Indication (color change):

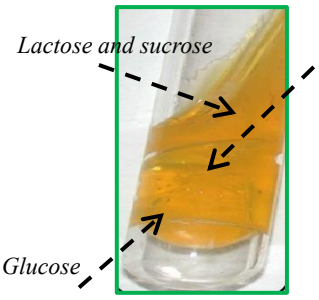
1 & 2: we have two compartments in the medium; yellow (A) → (+); red (K) → (-) A/A → (+) for both 1 & 2; K/A → (+) for only 1; K/K → (-) for both.

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

*** Situation (a): A/A**

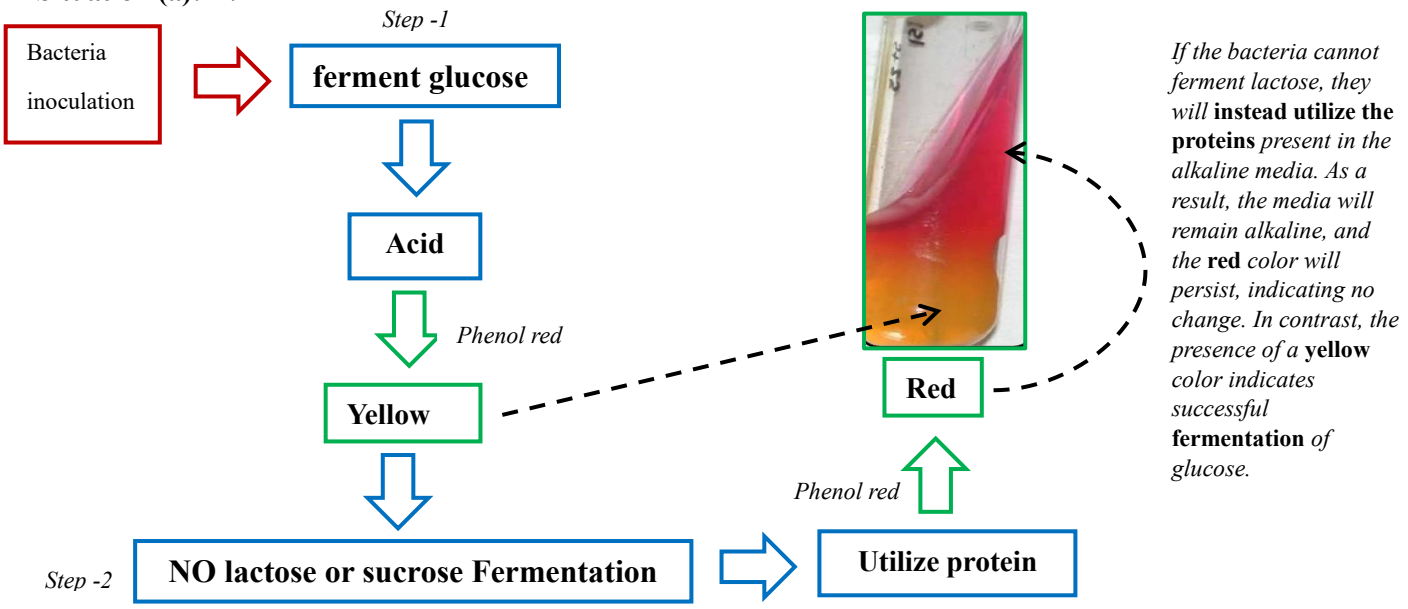
Bacteria inoculation → *Step -1* **ferment glucose** → **Acid** → *Phenol red* → **Yellow** → *Step -2* **Then lactose or sucrose Fermentation** → **Acid**



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

Yellow → *Phenol red* → **Acid**

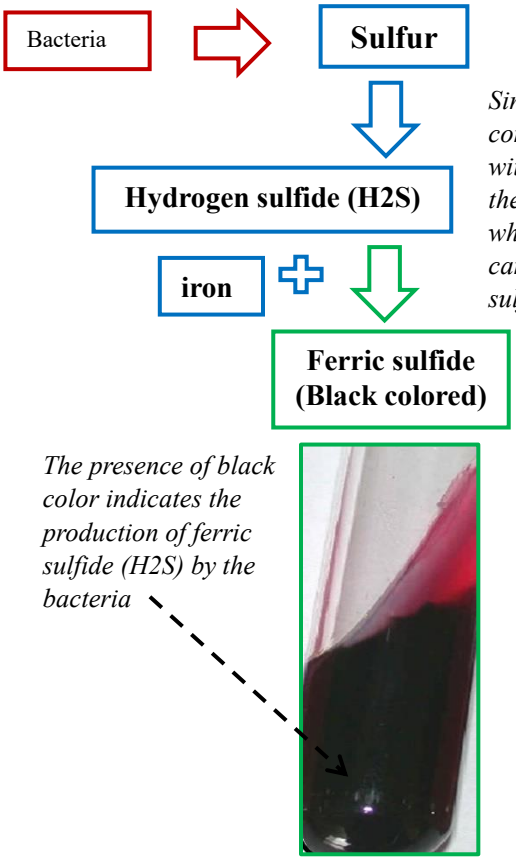
*** Situation (a): K/A**



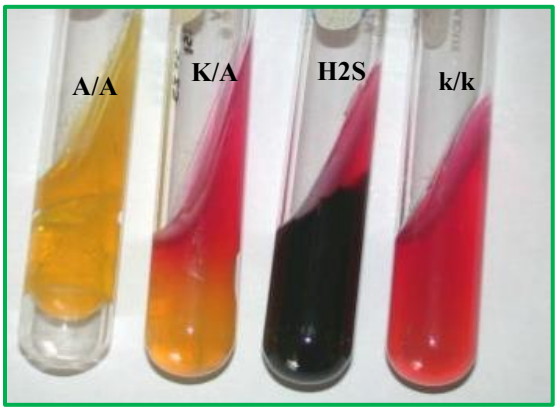
*** Situation (a): K/A**

- The bacteria is Neither a glucose fermenter nor a lactose/sucrose fermenter.
- If the organism cannot use the glucose in the medium. The color of the medium remains red.

***Situation (d): H2S production**



To sum up all results/ indications:



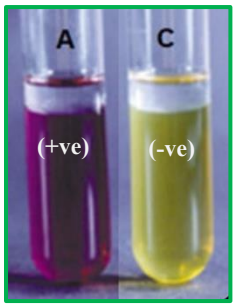
Note: glucose fermenter is always down, Lactose and sucrose is up…

8) Ornithine decarboxylase

Bacteria → *Step -1* **ferment glucose** → **Yellow**

Step -2 **Ornithine**

Ornithine decarboxylase → **Source of carbon (energy for growth)** → **purple**



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

Tests for: To distinguish between P. rettgeri & M. morganii.

How? The test determines whether bacteria can decarboxylate the amino acid ornithine and use it as a carbon source for growth. If +ve then it is Providencia rettgeri, since it has the ornithine decarboxylase enzyme. However, Morganella morganii lacks it

Indication (color change):

Purple (after initial yellow): (+ve) ornithine decarboxylase> M. morgani

Yellow (no color change back to purple): Negative for ornithine decarboxylase > P. rettgeri

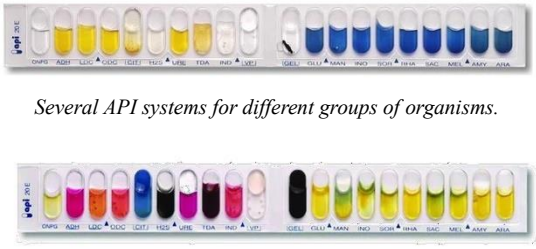
(Bromocresol purple (Indicator))

9) Analytical profile index (API)

Tests for: commercial stripe contains multiple tubes for various biochemical reactions > various tests

How? 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.

Example: determine which species of Streptococcus.

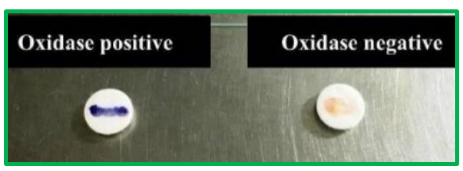


Several API systems for different groups of organisms.

10) Oxidase test

bacteria → **few drops of colorless Oxidase reagent**

Oxidase enzyme Detection → **Indophenol (Purple colored)**



Tests for: Used to differentiate Enterobacteriaceae from pseudomonas

How? All Enterobacteriaceae are oxidase- negative, except 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.

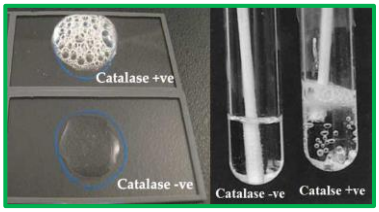
11) Catalase test

bacteria ⇨ **Addition of H2O2**

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

Gas bubbles

(O2 production)



Tests for: Used to differentiate between staphylococci and streptococci…since we cannot rely on morphology only. *Refer to the table*

How? staphylococci bacteria produce the enzyme **catalase**, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. However, streptococci lack it.

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

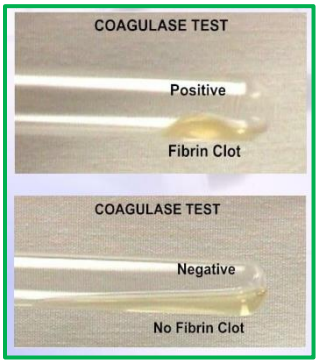
Characteristic	Staphylococci	Streptococci
Catalase Test	Catalase positive & Coagulase +ve.	Catalase negative
Morphology	Forms clusters	Forms chains
Gram Staining	Gram-positive	Gram-positive
Shape	Cocci (spherical bacteria)	Cocci (spherical bacteria)

12) Coagulase test

bacteria ⇨ **fibrinogen**

Incubate at 37°C for 24 hours ⇩ *coagulase enzyme detection*

fibrin (plasma clot)



Tests for: After confirming that it is Staphylococci, which are catalase-positive, we use a coagulase test to differentiate staphylococcus aureus from other species under the staphylococci genus.

How? detects the presence of the enzyme coagulase in bacteria, which enables them to clot plasma. This is used to differentiate Staphylococcus aureus (coagulase-positive) from other *Staphylococcus* species (coagulase-negative).

Indication (color change):

forming of fiber clot, indicate Coagulase-positive bacteria. Which is Staphylococcus aureus since it is the only member of the Staphylococcus family that is coagulase +ve.

➢ Vitek system *(More advanced than API)*

- **An automated system**

***Performs 3 several functions:**

1. **Identification …** *Can identify almost every microorganism.*
2. **Antibiogram**

> *Determines the susceptibility of bacteria to various antibodies, which is helpful in determining which treatment is effective for bacterial infections.*

3. **Antifungals**

> *Identifying and determining the susceptibility of fungal pathogens to various antifungal agents, to also decide the best approach to effective treatment.*

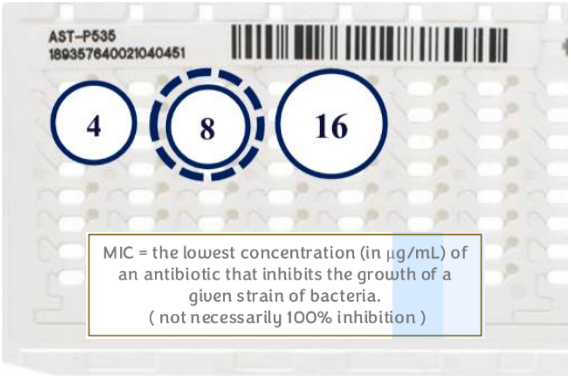
***Contains two cards:**

1) **Identification card (ID card)**:
   - To know the type of the bacteria or fungi (causative agents)
   - 47 biochemical tests… *while in a routine lab, we can barely get 10 biochemical tests*.
   - Specific Card for GN (Gram -)
   - Specific Card for GP (Gram +)
   - Specific Card for Yeast

2) **Antimicrobial susceptibility test card (AST card)**
   1. 22 antibiotics… *Gives us all the antibiotics that we can use effectively for the patient.*
   2. MIC Minimum Inhibitory Concentration of antibiotic.

> Some patients suffer from certain infection, but also have kidney problems, for example. In this case, we don't want to give them a strong antibiotic. So, we look for the MIC of this antibody, which will help the patient while inflicting the least amount of damage. It allows for **personalized treatment for the patient by choosing the most effective drug concentration** that is compatible with the patient's kidney function and overall health.

*Process of Determination of the minimum inhibitory concentration (MIC) of an antibiotic for a specific bacterium:



1. There are **64 wells** present. Every well has a different antibiotic concentration. The first well has an antibiotic concentration of 4 µg/mL. The second has 8 µg/mL. The third has 16 µg/mL, and so on.
2. First, the **4** µg/mL is observed. The bacteria is still alive and active at this antibiotic concentration.
3. Then, we observe the **8** µg/mLwell. It's apparent that this antibiotic concentration was 80% effective.
4. However, when we observe the **16** µg/mLwell, we can see this antibiotic concentration was 100% effective against this bacterium. **Thus, the MIC is determined to be 8µg/mL of this antibiotic.**

* Vitek system steps of work:

1) Organism isolation (Pure).

These appearing colonies must be pure and uncontaminated, because we are trying to determine the causative agent responsible for this disease. Then, I take these colonies and produce two bacterial suspensions.

2) Bacterial suspension (2 tubes).

This suspension forms turbidity, which refers to the cloudiness caused by the bacteria's presence. More turbidity = more bacterial growth. This turbidity is a result of the inoculation, or the addition of the colonies to a growth medium (growth broth), which promotes the bacteria's growth. This turbidity should follow a certain standard. Otherwise, if the bacterial growth is exceptionally high, for example, the antibiotics are not going to present their effectiveness fully, which may lead us to think that this antibody is ineffective against this bacteria, when it actually is →false negative. On the other hand, if the bacterial growth was very low, the antibody will present exaggerated effectiveness against this bacteria, when its effectiveness is less →false positive.

3) Measure turbidity (0.5 -0.63).

The turbidity standard (McFarland Standards)

4) Insert cards (ID & AST) in bacterial Suspension tubes.

5) Into the filling room Transfers the bacterial suspension into the wells.

Two patients can have the same pathogen (IDs), and still have different AST results because ASTs are personalized and differ from one patient to another (different resistance).

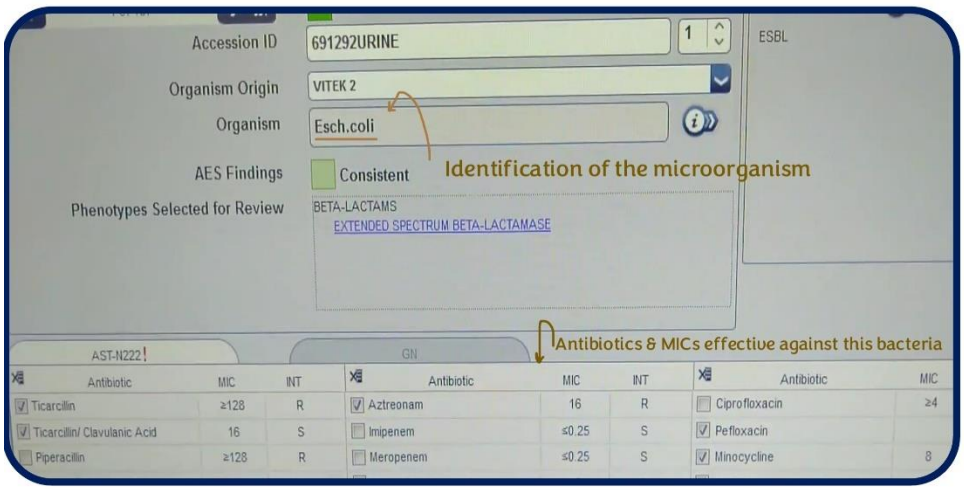
6) Transfer the cassette into the loading room (Diagnostic) 5-10hrs …*Compared to API's 24 hours.*

recall:

7) Colorimetric (Barcode).

This barcode reads biochemical tests, color changes, metabolism, etc. and then compares this data to its stored database of almost every microorganism. If it detects at least a 90% similarity between the scanned and stored data, it identifies the microorganism and the most effective antibiotic treatment.

8) Identifications according to the result.

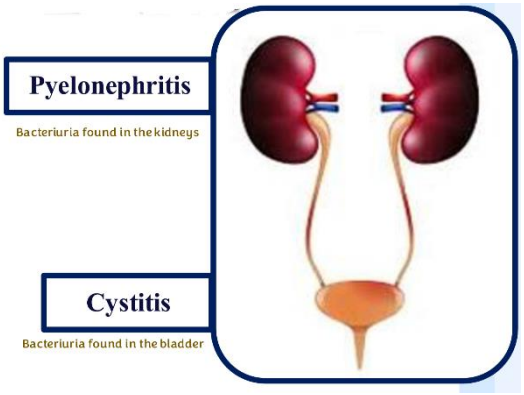


➢ Urine culture technique

***Purpose of Urine culture technique:**

- To diagnose Urinary tract infection (UTI) which signifies the presence of bacteriuria.

The urinary tract (more specifically, the bladder and organs above) in healthy individuals is sterile and should contain no microorganisms. Microorganisms present in the urinary tract are called bacteriuria.



UTI ⇨ **Bacteriuria** ⇨ *Resulting in:*

Dysuria: Burning sensation present during urination

Symptoms of bacteriuria:

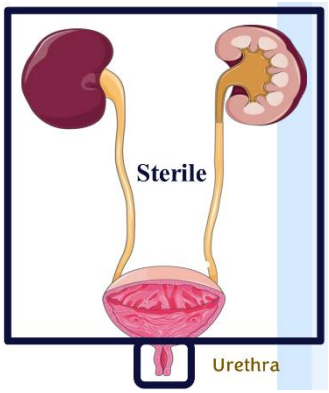
Frequency: Lots of bathroom trips

*** UTI Confirmation >>> Bacterial count:**

UTI ⇨ **Bacteriuria** ⇨ **Significant:** if **Significant amount** *is reached*: **$\leq 10^5$CFU/ml**

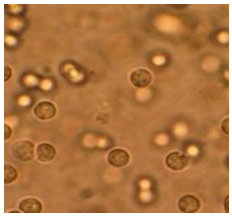
- *A significant amount of bacteriuria must be present for UTI conformation which must be* **$\leq 10^5$CFU/ml**
- *Colony forming unit (CFU)*
- *When we culture bacteria, we make sure it divides 20-30 times to produce a lot of colonies. If it produces ~100,000 colonies, we can label it as significant bacteriuria.*

The urethra is the site of contamination, serving as entry point for bacteria. The urinary tract contains normal flora, but if harmful microorganisms interact with it, it can lead to the formation of bacteriuria. If the bacteria travel up to the bladder it causes cystitis, and if it reaches the kidneys, it can lead to pyelonephritis.



Pyuria

Finding cells in urine



⇨ **(Pus in urine >10 cells/HPF** High power field**).**

Pus signifies an inflammatory response to infection. It consists of WBCs, dead tissues, and bacteria. This pus is thick and discolored (white, yellow, pink, or green). The counted cells in pyuria are WBCs (leukocytes)

⇨ **Significant Bacteriuria**

Bacteriuria and pyuria are often found together.

***Types of Specimens** > *taken from patients*:

Mid-stream urine

Catheterization (قسطرة)

Urine sample collected by a catheter

A catheter is a thin, flexible tube inserted into the body to allow the passage of fluids.

Suprapubic aspiration

The specimen is taken directly from the bladder, especially for infants since they cannot control themselves to provide the required sample.

* How to collect Mid-stream urine?

Note: If patients are taking antibiotics, they should stop the antibiotics for 3 days.

because antibiotics can kill the bacteria, preventing successful culture growth.

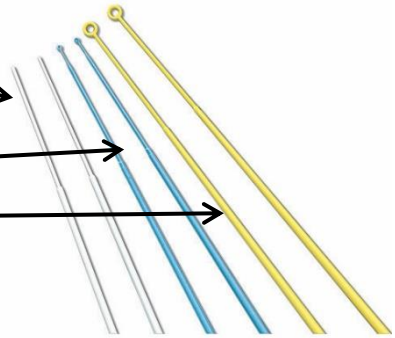
1) Wash and dry your hands.
2) Clean genital area.
3) Remove the lid on the container (Sterile).
4) Pass a small amount of urine into the toilet… *(at morning) > because it's concentrated.*
5) Mid-stream urine.
6) Pass the reimaging urine into the toilet.
7) Ready to be taken to the lab as soon as possible.

* Mid-stream urine method:

In the culturing process, an inoculating needle (pointed) is typically used. However, for urine cultures, a calibrated loop is preferred.

There are two types of calibrated loops:

1. **1 µL (0.001 mL)**: recommended for more precise inoculation.
2. **10 µL (0.01 mL)**



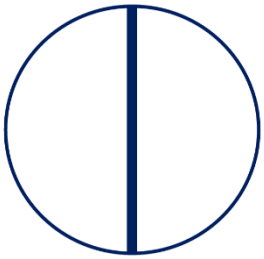
1) Mix urine (uncentrifuged) & by Calibrated loop

It can determine the volume taken from the sample to the culture plates; which is necessary for bacterial counting.

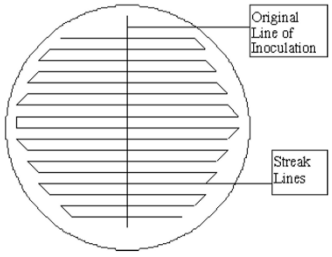
2) Inoculation on by streaking & incubate at 37 For 24hrs.

Urine culture is different from other cultures due to bacterial counting :

1.Draw a central Line (original line)



2. Zigzag (streaking line)



Two types of media:

3) **Examine centrifuged urine (≥ 10 cells/HPF) Pyuria**

centrifugation -> Examine under the microscope -> counting cells -> more than 10 cells = pyuria.

4) *Next day…* **Count the growth colonies.**

5) **Multiply the count by dilution factor.**

- If calibrated loop = **10µL (0.01ml)** → No. of colonies X **100** = 10^5CFU/ml
- If Calibrated loop = **1µL (0.001ml)** → No. of colonies X **1000** = 10^5CFU/ml

Further explanation

$$\frac{\text{CFU}}{\text{mL}} \quad \text{Volume} \qquad \text{CFU} = \text{colony-forming units} = \text{no. Of colonies}$$

Example:

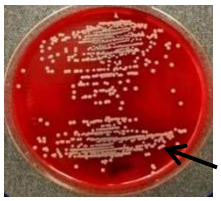
No. Of colonies \ 0.01 = CFU x 100

10 x 100 = 10^3 (Dilution factor)

6) **Interpretation: Analysis of results**

- General situation:

≤ 10^5CFU/ml → **Significant Bacteriuria** → **Identification** + *(AST)*



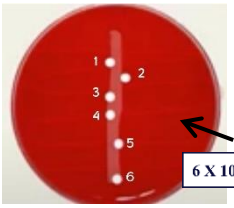
Obviously, UTI due to the presence of many colonies

- Exceptional situation: **ONLY IN CASE OF "Staph. Aureus"**

≤ 10^4CFU/ml

≤ 10^3CFU/ml

→ **Significant Bacteriuria** → **Identification**

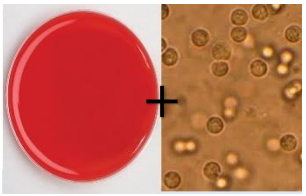


Given that: This bacteria is Staph. Aureus

6 X 1000= 60000 CFU/ml =10^4

- Situation (3): **Sterile pyuria**

Pus without any bacterial growth in ordinary media → **No microorganisms present**



= no UTI

*Causes of Sterile pyuria

1) Taking antibiotics = Microorganism's will be eliminated
2) Renal tuberculosis Responsible of the Pus (cells)
3) Renal stones Responsible of the Pus (cells) = there's no bacterial infection
4) Organism does not grow on {ordinary media = nutrient media = petri dish}.

Needs different, specific media:

- Mycoplasma
- L-form bacteria
- Anaerobic infection

﴿ رَبَّنَا أَفْرِغْ عَلَيْنَا صَبْرًا وَثَبِّتْ أَقْدَامَنَا وَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ ﴾

- Situation (4): **10^3 + pyuria?**

1) 10^3 > No (UTI) Although there is pyuria.

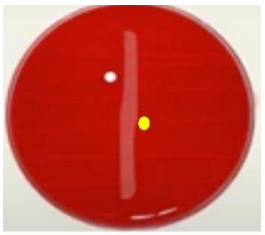
The reason a bacterial count of 10^3 *CFU/mL, along with pyuria, does not automatically mean a UTI is because pyuria can be caused by a variety of conditions such as:*

1) **Prostatitis** (Male with Inflammation of the prostate gland)
2) **Vaginitis** (Female Inflammation of the vagina)
3) **Cervicitis** (Female Inflammation of the cervix)
4) **Malignancy**
5) **Renal calculi** = renal stones

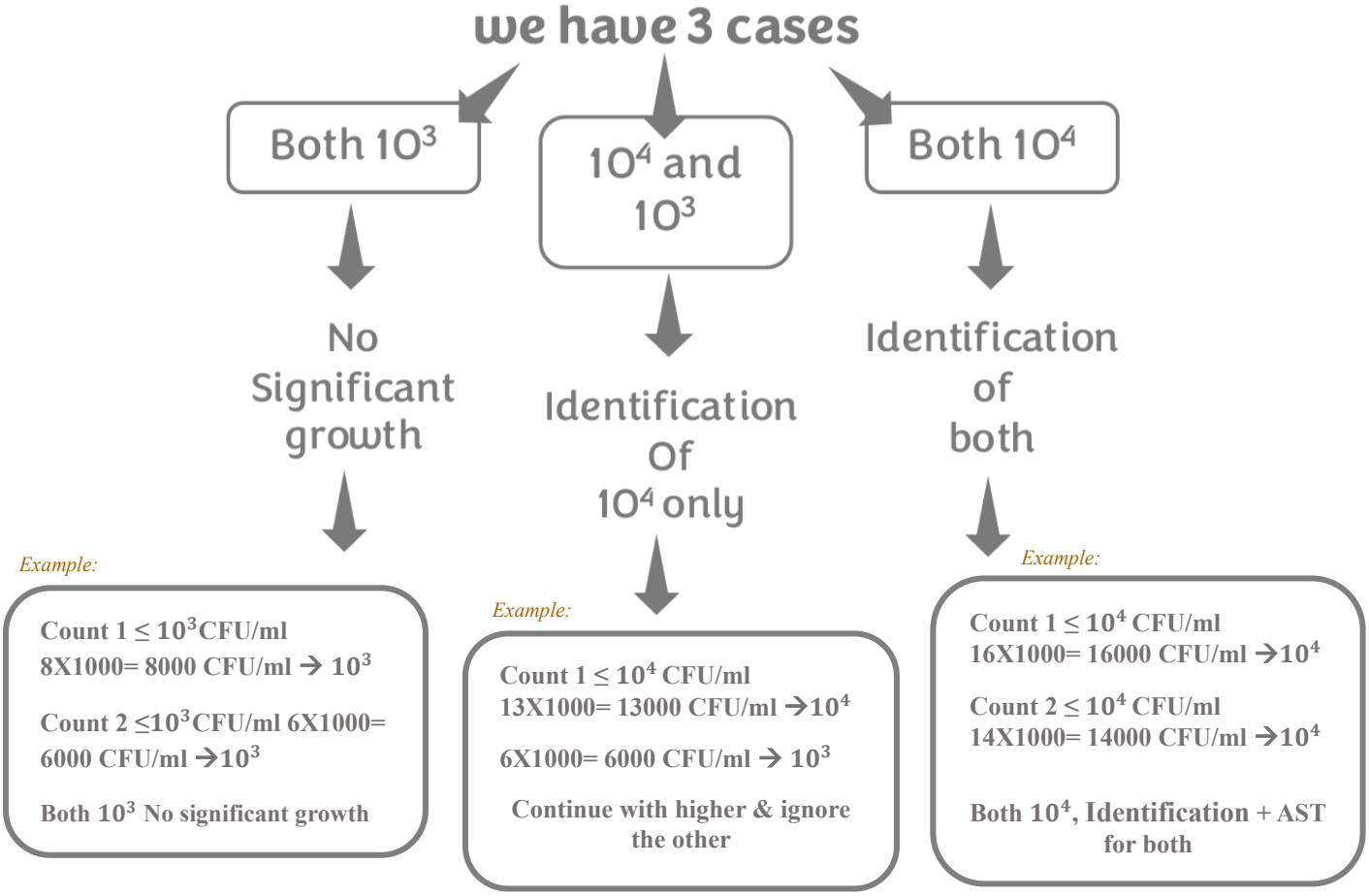
If you are thinking about Staph. Aureus case!

staph aureus (golden in Latin) can be Primarily identified macroscopically from its golden color \cocci shape \ G+

- Situation (5): **In case of Suprapubic aspiration & Catheterization.**
  - Any growth is significant bacteriuria
  - even in the presence of a single colony
  - Because it's directly taken from the bladder = there's no contamination
  - 1X1000= 1000 CFU/ml = 10^3 → UTI



*** Urine culture Interpretation - Two pathogen:**



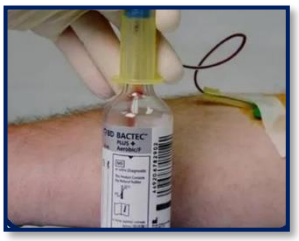
➢ Blood culture

***Purpose:**

- To check if there's Bacteremic infections… *{bacteremia= a pathogen or microorganism in blood}*
  Causing different diseases: **1-Typhoid 2- Fever 3-Endocarditis 4-Puerperal sepsis 5-Brucellosis.**

***Specimen:**

1) 3ml blood to 30 ml broth for child BACTEC system
2) 10 ml blood to 30 ml broth for adult (aerobic)
3) 10 ml blood to 40 ml broth for adult (anerobic) - *Blood draw procedure occurs under septic conditions.*



BACTEC tube contains nutrient broth

***Purposes of the broth:**

- Dilutes antibacterial antibodies
- Provides good nutrient (organism present in small number) > to increase their number.

***Purposes of culture (recall):**

1. **Detection Bacteria**

   Bacteria present = bacterial infection

   No bacteria = no bacterial infection

2. **Identification**

   Identify the causative agent ↞that's why we grow them

3. **AST**

***Method:**

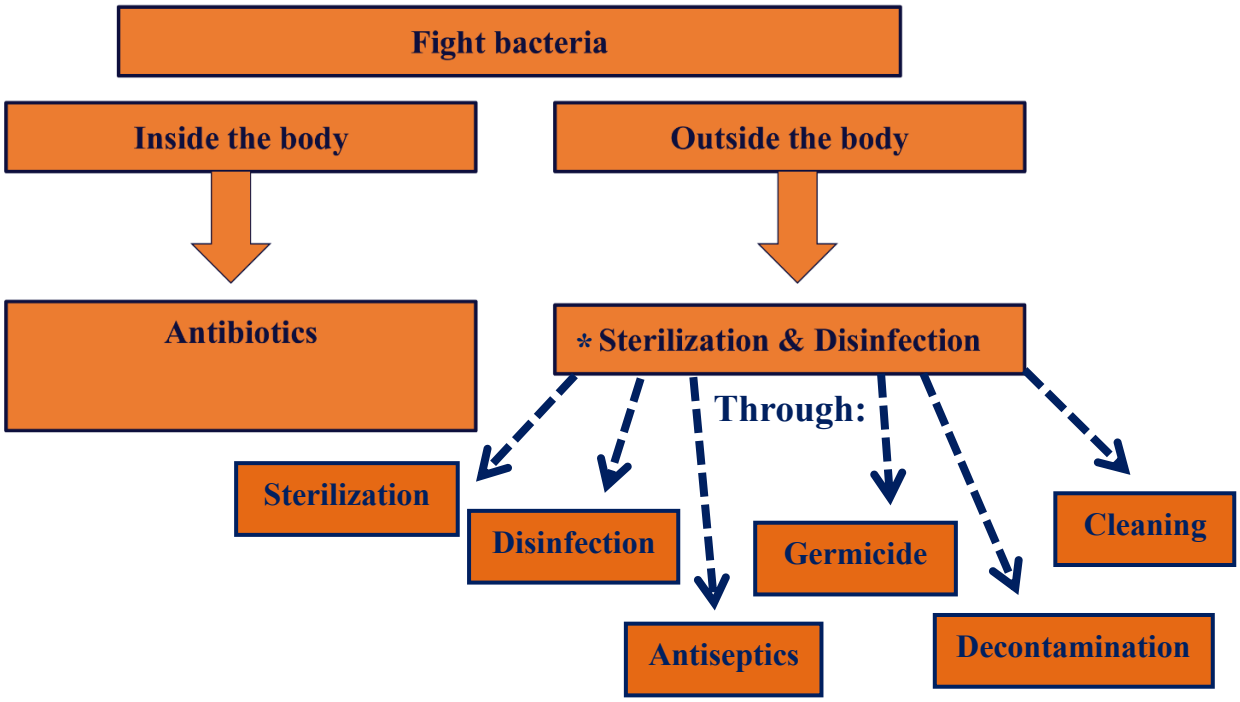
1) **Incubation 5 to 21 days**

- Specially in brucellosis
- Very long period… in this case we should prioritize the patient's life and give an antibiotic even if there was no bacteria (AB is abused) As the benefits outweigh the risks.

2) **Organism present → Consume nutrients → CO_2 released → CO_2 reacts with sensor → Light appears in the bottle.**

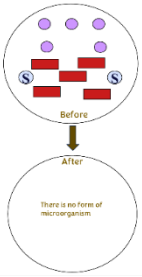
3) **Then we should Sub culture & incubate at 37°C for 24h → Identification → Susceptibility test.**

To fight the bacteria, we need to determine its location



➢ Sterilization:

*Definition of Sterilization:



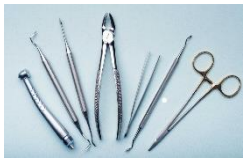
- Removal or killing of all forms of living microorganisms including bacterial spores physical or chemical methods.
- Absolute term Killing or removing All Microorganisms.

'sterile', 'sterilization'> all forms of bacteria have been killed.

*Uses of Sterilization:

Surgical instruments

The surgical instruments must be sterile, with no bacteria or spores present.



Syringes, gloves, and catheters

They must be sterilized because they come into direct contact with patients, especially syringes and catheters, which are inserted into the body.

Bacteria Culture

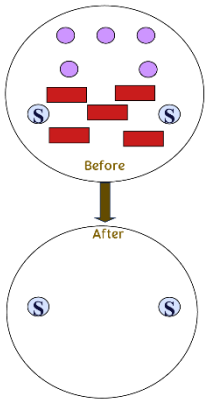
The process seeks the pathogen responsible for a disease. requiring a sterile, uncontaminated culture medium. If not sterilized, the investigation will yield inaccurate results.

*Methods of Sterilization:

Physical methods:	Chemical methods:
Heat	Gaseous
Radiation	Liquids
Filtration	

➢ Disinfection:

*Definitions:



- Disinfection Removal of most (if not all) pathogenic organisms except bacterial spores by physical or chemical methods.
- Disinfectants: Chemical substances that are used to achieve disinfection.

The difference between disinfection and disinfectants: disinfection refers to the process itself. while disinfectants are the chemicals used to achieve disinfection > usually toxic.

*Levels of Disinfection:

A. **High level disinfectants**

- Kill all microbes EXCEPT large number of bacterial Spores.
- e.g. H2O2 For contact lens

High level disinfectants can approach sterilization-level effectiveness, especially when only a small number of spores are present, but it typically does not guarantee the complete elimination of all spores as sterilization does.

B. **Intermediate level disinfectants**

- Kill all microbes EXCEPT Bacterial Spores.
- e.g. alcohol

C. **Low level disinfectants**

- Kill MOST vegetative Bacteria EXCEPT Mycobacterium tuberculosis.

We can use Mycobacterium tuberculosis as an indicator of disinfectant efficacy. For example, if we have a disinfectant and want to assess its effectiveness, we can introduce this bacteria to it, as M. tuberculosis is resistant to low-level disinfectants. If the bacteria survive, it suggests a low-level disinfectant, while if it is killed, it suggests an intermediate or high-level disinfectant.

*Methods for disinfection:

Physical Methods

1) Moist heat
2) Radiation

Chemical Agents

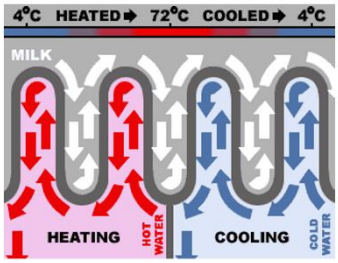
* Physical methods for disinfection

I. Moist heat >>> *Moist heat is divided into three levels*

i. **Below 100°C - (pasteurization)**

- Pasteurization > is considered as disinfectant, since it can get rid of some bacteria but not all.
- Should be heated at: 63°C for 30 min. or 72°C for 20 sec.
- {Heat →→→ Cooling} immediately

The pasteurization process must be followed by rapid cooling; if high temperatures are maintained, it will create an appropriate environment for thermophilic bacteria to grow. In the past, milk and juice factories used pasteurization to prevent contamination, but it's now considered inefficient due to its 2–3-day process. Today, autoclaving is used instead.



Not sterilizing, Kills:

- *M. tuberculosis*
- *B. abortus… causes Brucellosis*
- *Salmonella … causes Typhoid fever*
- *C. burnetii… Q fever*

ii. **Boiling at 100°C**

- Boiling (100°C) for 20 min.

*Use cases:

- Kills all vegetative bacteria
- In emergency

*Equipment to be boiled:

- Glass Syringes
- Surgical instrument

The boiling method was used in hospitals in the past, but today it is only employed in emergencies. It is important to note that the 20-minute countdown begins only after the water starts boiling.

iii. **Above 100°C**, also known as autoclaving or steam sterilization, with temperatures often reaching 121°C or higher.

II. Radiation

- Ultraviolet rays
- it is disinfection, not sterilization, due to: Low Ultraviolet rays' penetration & Surface disinfectant.
- Artificially by mercury lamps
- (advantage): Bactericidal … *kills bacteria*
- (disadvantage): Carcinogen… *causes cancer often used in empty surgery rooms.*

used in:

- Operation room… Applied only after all personnel have left the room
- Drug filling cubicles
- Safety cabinets.

* Chemical methods for disinfection: (low – intermediate – high)

Q: Why is there resistance to antibiotics and usually no resistant for chemical disinfectants?

Ans: Because Chemical disinfectants have a combination of actions **Oxidation, Denaturation, Breaks DNA, Cell membrane & cell wall damage.** While each antibiotic has a single target like: ribosomes, cell membrane or NA.

I. Low level disinfectants

- Kills MOST microbes, EXCEPT TB & bacterial Spores.
- Example: (Quaternary Ammonium Compounds) – such as: Benzethonium Chloride & Benzalkonium chloride.
- Used in: Disinfection of Floors & Blood spills.

II. Intermediate level disinfectants

- kills most (all) microbes except bacterial spores.

i. Alcohols 70%

It's more efficient at 70% because when it's diluted by water its penetration ability increases, while 100% concentrated alcohol shocks the bacteria initially making it rapidly resistant, so 100% alcohol fails to kill microbes effectively.

- **Act as:**
  - Bactericidal … kills bacteria
  - Fungicidal … kills fungi
  - Virucidal … kills viruses (specifically the enveloped ones)
- **Mechanism of killing:**
  - Membrane damage
  - Denaturation
  - Disruption of lipid containing
- **Examples:**
  - Ethanol (Ethyl alcohol)
  - Isopropanol (Isopropyl alcohol) … more stable
  - Methanol (Methyl alcohol)

    Even just sniffing methanol can cause extensive damage, including blindness, brain damage, and death.
- **Used as:**
  - Antiseptic
  - hand sanitizer

ii. Phenols:

- First used in the operation room by Lister.
  - **Mechanism of killing:**
    - Membrane damage
    - Denaturation
  - **Examples // derivatives:**
    - Cresol (Lysol)
    - Chloroxylenol
  - **Used as:**
    - Used for floors.
    - Culture spills… If any culture dish fell off and got contaminated.

iii. Biguanides:

- **Examples:**
  - Chlorhexidine
- **Used as:**
  - Antiseptic (mouth washing)

iv. Halogens:

- **Mechanism of killing:**
  - Denaturation
  - Oxidation
- **Examples:**
  - Iodine:

*Tincture iodine: (2% Iodine + 2.4% sodium iodide in 50% ethanol) **Used as:** Skin antiseptic

*Betadine: (povidone + iodine) >> Stronger than Tincture Iodine. **Used as:** Skin antiseptic

  - Fluorine:
    *** Used as:** Toothpaste
  - Chlorine:
    It is a high-level disinfectant but is included here because it belongs to the halogen family.

v. Heavy metals

- **Mechanism of killing:**
  - Denaturation
  - Enzyme activity inhibition
- **Examples:**
  - **Nickel**
  - **Copper**
  - **Zinc**:

Zinc (Zinc oxide)
• Calamine lotion > To relieve the itching for chickenpox patients.
• Baby powder

  - **Silver**:

- **Used as:** drinking water was stored in silver jugs
- Silver nitrate drops **(ophthalmia neonatorum)**- if a pregnant woman is infected with gonorrhea, the newborn can become infected during birth, leading to a condition called ophthalmia neonatorum. While silver nitrate drops were once used for prevention, this condition is now typically treated and prevented with antibiotic eye ointments, such as erythromycin.

- **Used as:**
  - **Nickel / Copper & Zinc** act as antimicrobial activity > reduces microbes.
  - Advantage: Some modern hospitals utilize **Nickel / Copper & Zinc** to manufacture doorknobs in order to decrease contamination and control infections.
  - Disadvantage: Toxic to human & animal in excessive concentration (**Argyria**)… *A blue discoloration on the patient hands and face as an effect of high concentrations of heavy metals.*

III. High level disinfectants:

- Kills all microbes except large numbers of bacterial spores.
- Equivalent to sterilant while dealing with low numbers of bacterial spores as it can handle it.

- **Examples:**
  - **Chlorine**:
    - **Used in:** in swimming pools & water
    - Sodium Hypochlorite: (Chlorine + Sodium + Oxygen)
      - Disinfectant in homes & hospitals
      - Disadvantages: 1- Bleaching effect (ruining colors).
        2- Corrosive (if we use it for metals).
  - **Hydrogen peroxide**

    * Act as Antiseptic
  - **Glutaraldehyde 2%**
  - **Peracetic acids**

    - Needs ~10 hours

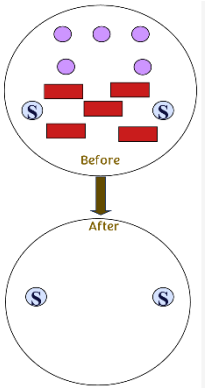
    It depends on the contact time:

    - ~20 minutes is considered a normal disinfectant.
    - ~10 hours are considered a high-level disinfectant or sterilant.

TO SUM UP: VERY IMPORTANT!

Level	Agent	Effect	Use Cases
Low-Level	Quaternary Ammonium Compounds: 1.Benzalkonium Chloride, 2.Benzethonium Chloride	Kills most microbes except Mycobacterium tuberculosis and spores	**Disinfection of floors, blood spills**
Intermediate-Level	Alcohols (70% Ethanol, Isopropanol)	Bactericidal, fungicidal, virucidal (enveloped viruses)	**Hand sanitizers, antiseptics**
	Phenols (Chloroxylenol, Cresol)	Membrane damage, protein denaturation	**Disinfection of floors, culture spills**
	Chlorhexidine (Biguanides)	Antiseptic	**Mouthwash**
	Halogens (Fluoride, Iodine)	Oxidation, denaturation	**Skin antiseptics, Toothpaste**
	Heavy Metals (Copper, Zinc)	Enzyme inhibition, protein denaturation	**Doorknobs, calamine lotion, baby powder**
High-Level	Chlorine (Sodium Hypochlorite)	Kills all microbes except large numbers Of bacterial spores	**Water treatment, hospital disinfection**
	Hydrogen Peroxide		**Antiseptic**
	Glutaraldehyde (2%) and Peracetic Acid	Disinfectant, sterilant (based on contact time)	**High-level disinfectant, sterilant**

➢ Antiseptics:



- Antiseptics Removal of most (if not all) microbes Except bacterial spores.
- Non-TOXIC.

Antiseptics have the same effect as the disinfection process, but the difference is: Antiseptics are non-toxic materials (so it can be applied to living tissue) while disinfectants are toxic. لذلك يمكن استخدامه لتطهير الجروح

➢ Germicide:

- Germi → microbe… cide → killer
- Agent destroys microorganism.

*** Categories:** *>> based on the type of microbe that it is killing.*

- **Virucide**
- **Bactericide**
- **Fungicide**

***When an Agent destroys microorganism, it can act as:**

I. Disinfectant
- **-** kills most bacteria, but spores may still be present.
- We refer to the Germicide as a Disinfectant, when it achieves disinfection.

II. Antiseptic
- kills most bacteria, but spores may still be present.
- We refer to the Germicide as an Antiseptic, when it is non-toxic and achieves disinfection.

III. Sterilant
- All living microorganisms are killed, including spores.
- We refer to the Germicide as an Sterilant, when a Chemical germicide achieves sterilization.

➢ Cleaning:

- Removal of foreign material from medical devices by water & soap.
- Precedes disinfection & sterilization.

➢ Decontamination:

- Reduction of organisms to a level at which items are safe to handle.

It is not logical to perform sterilization or disinfection when the tools are contaminated.

***Include:**

- Cleaning >> Water & soap
- Disinfection >> some spores left
- Sterilization >> killing all microorganisms, spores included.

DOPAMINE SCIENTIFIC TEAM

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