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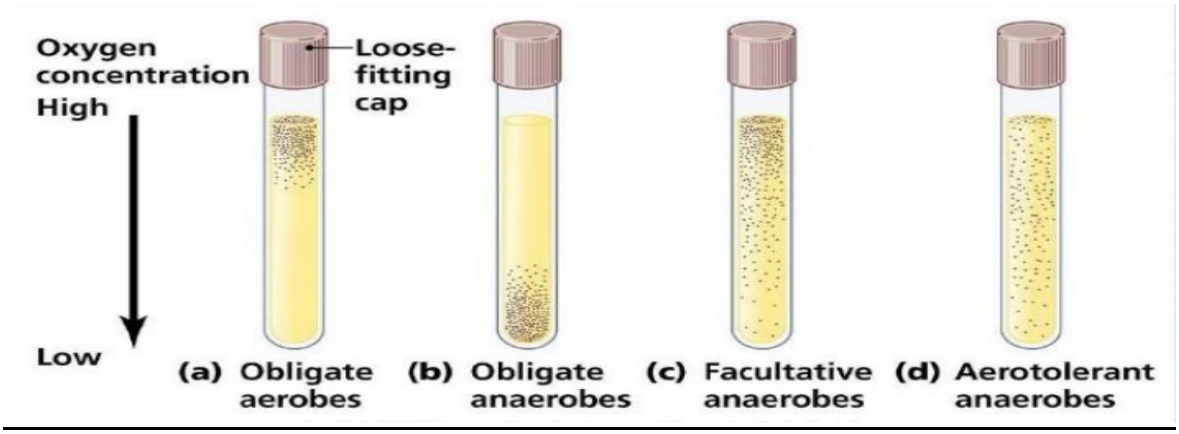
The Effect of Oxygen on Bacterial Growth:

1. **Obligate aerobes ex: TB**
2. **Facultative anaerobes ex: E-coli**
3. **Obligate anaerobes ex: clostridium tetani**
4. **Aerotolerant anaerobes**
5. **Micro-aerophiles**

الجدول جدا مهم بيشرح كل اشي اله علاقة بالاوكسجين والبكتيريا مهم جدا

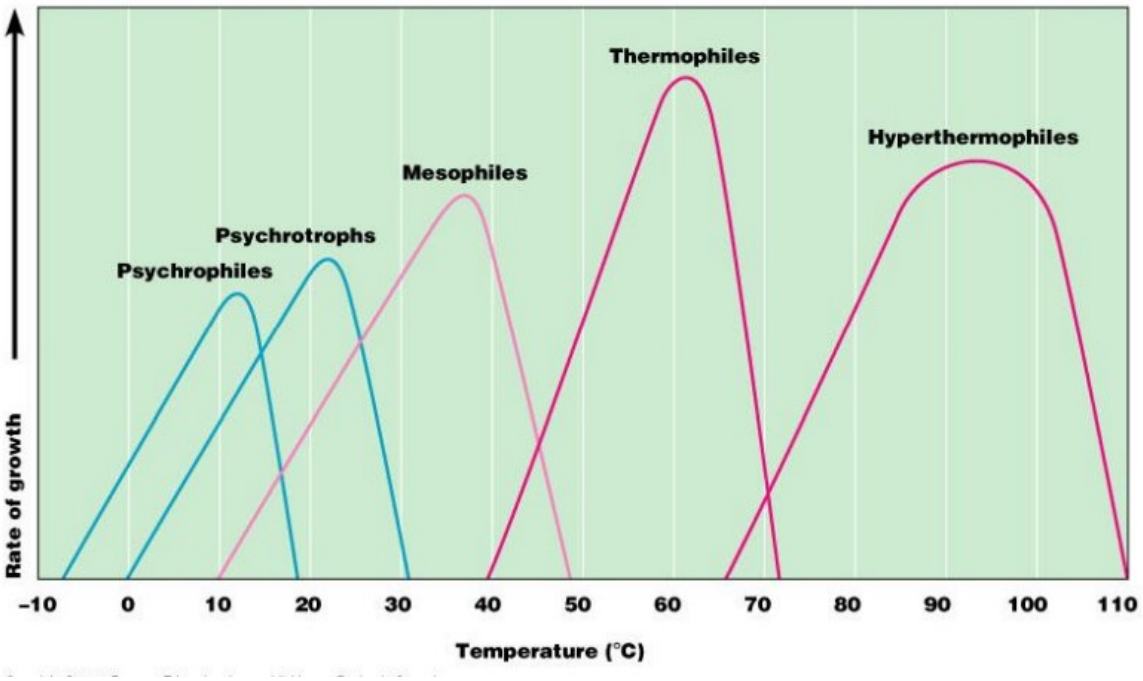
TABLE 6.1 **The Effect of Oxygen on the Growth of Various Types of Bacteria**

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Micro-aerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

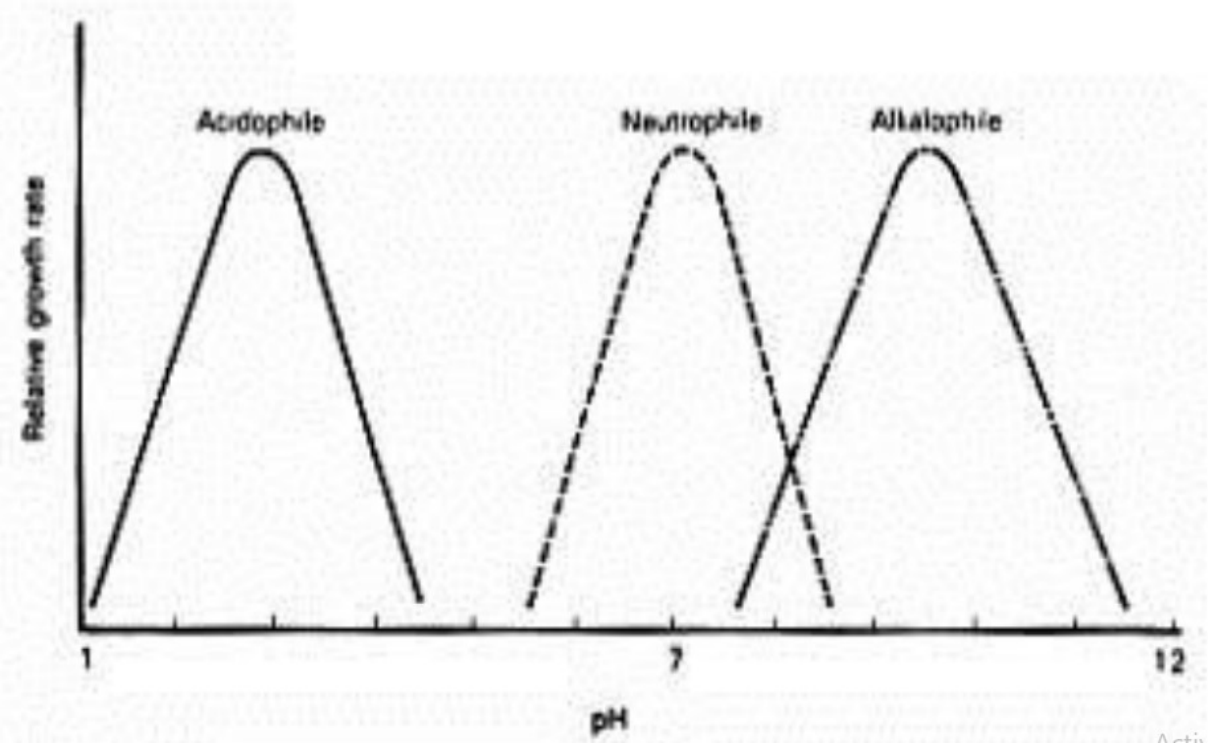


Classification according temperature :

The majority of bacteria is mesophilic:

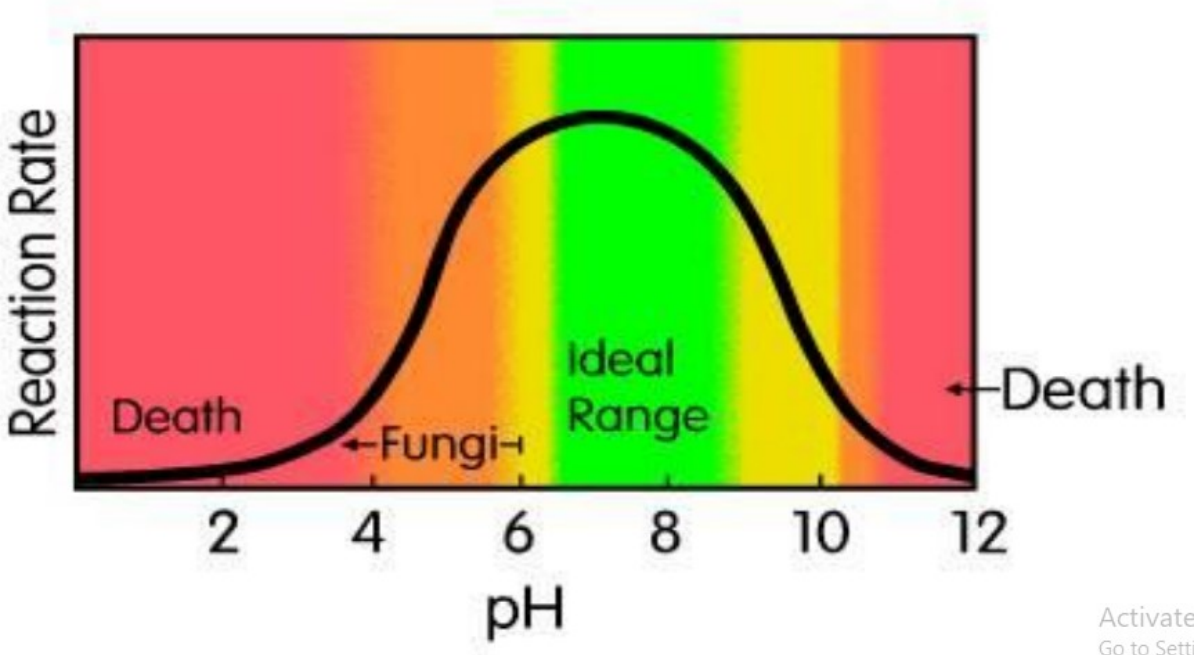


The Effect of pH on Bacterial Growth



In human cells the suitable medium for bacterial growth is neutrophile.

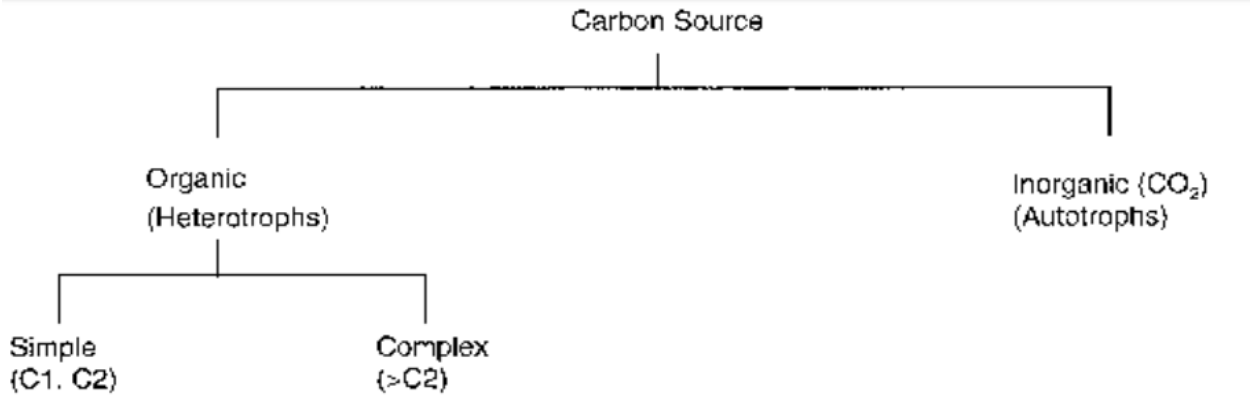
Acidophilic examples: in stomach (قرحة) + bacilli in vaginal wall (precipitation of lactic acid).



The Effect of Pressure on Bacterial Growth

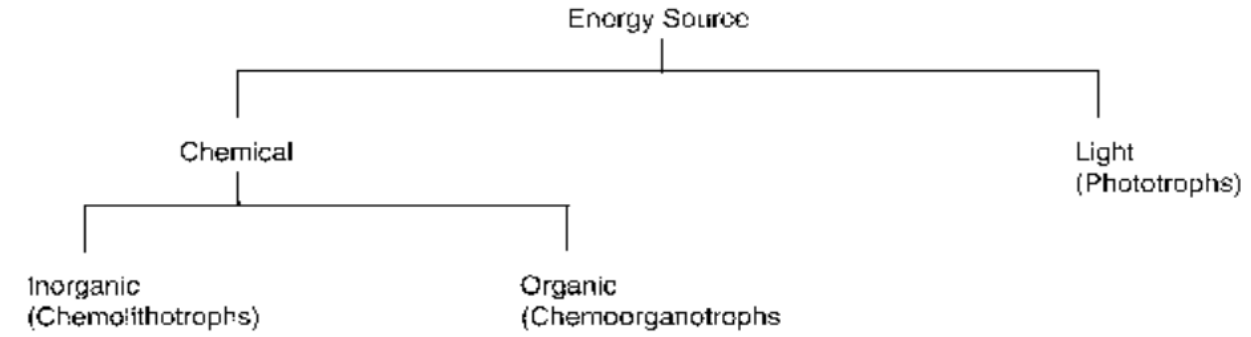
- Halophilic: organisms requiring high salt concentrations
- Osmophilic: organisms requiring high osmotic pressure- especially in human cells

Classification by nutritional requirement



Autotrophs (self-feeding): using a single carbon source.

Heterotrophs: using carbon from other organisms to obtain energy (pathogenic parasite).



Photoautotrophs: ex: (cyanobacteria), which synthesize foods using light energy and carbon dioxide gas and a single carbon source (photosynthesis). Right organ compound (complex like fatty acids).

Photo-heterotroph, light + organic compound fatty acid or alcohol (carbon source)

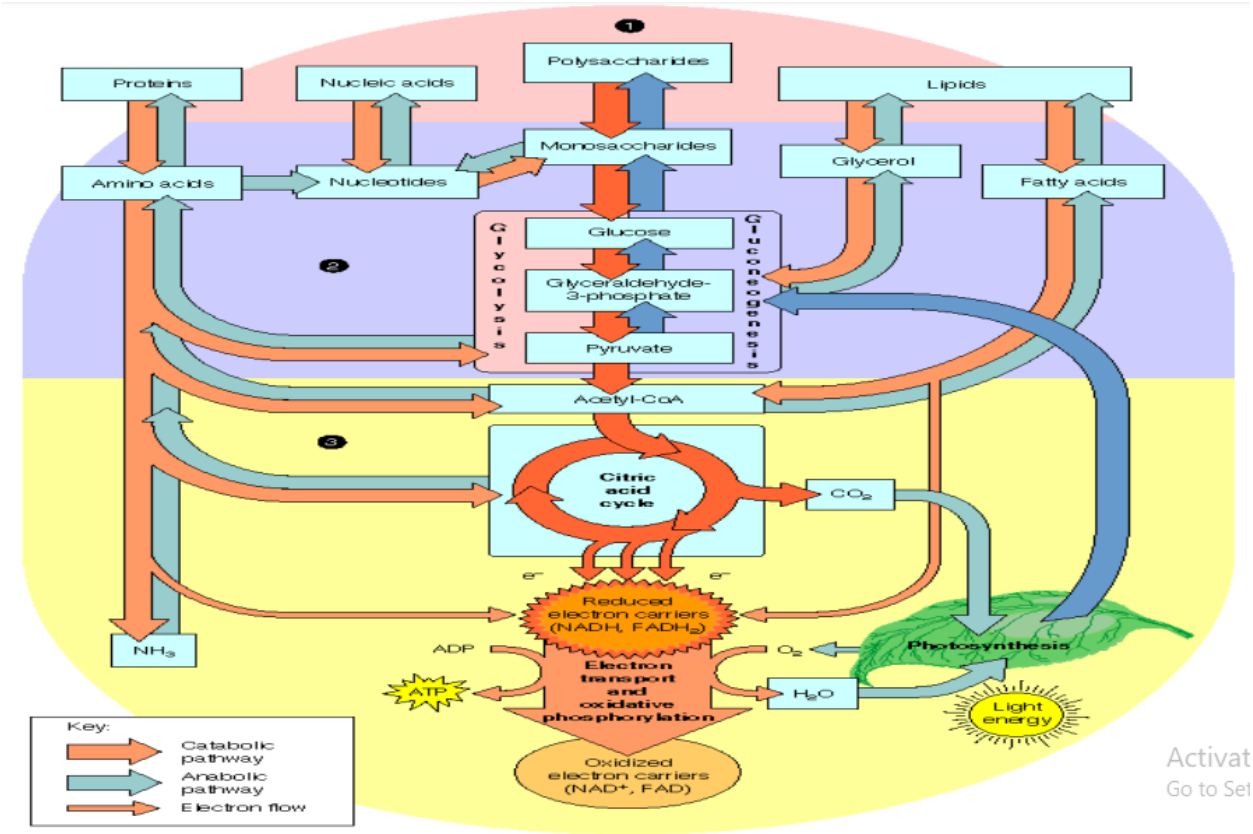
In **chemoautotroph** there is chemo-synthesis (CO_2 +chemical rxn) chemical reduction, for example lactose to produce energy, for the nutrition system (like parasites feeding on living organisms).

Chemo-heterotrophs, organic compounds for energy and carbon (glucose).

Metabolism, (change): comprised of anabolism and catabolism, it is the totality of an organism's chemical processes to maintain life.

Anabolism: building glycogen from glucose, takes up energy (endoergic)

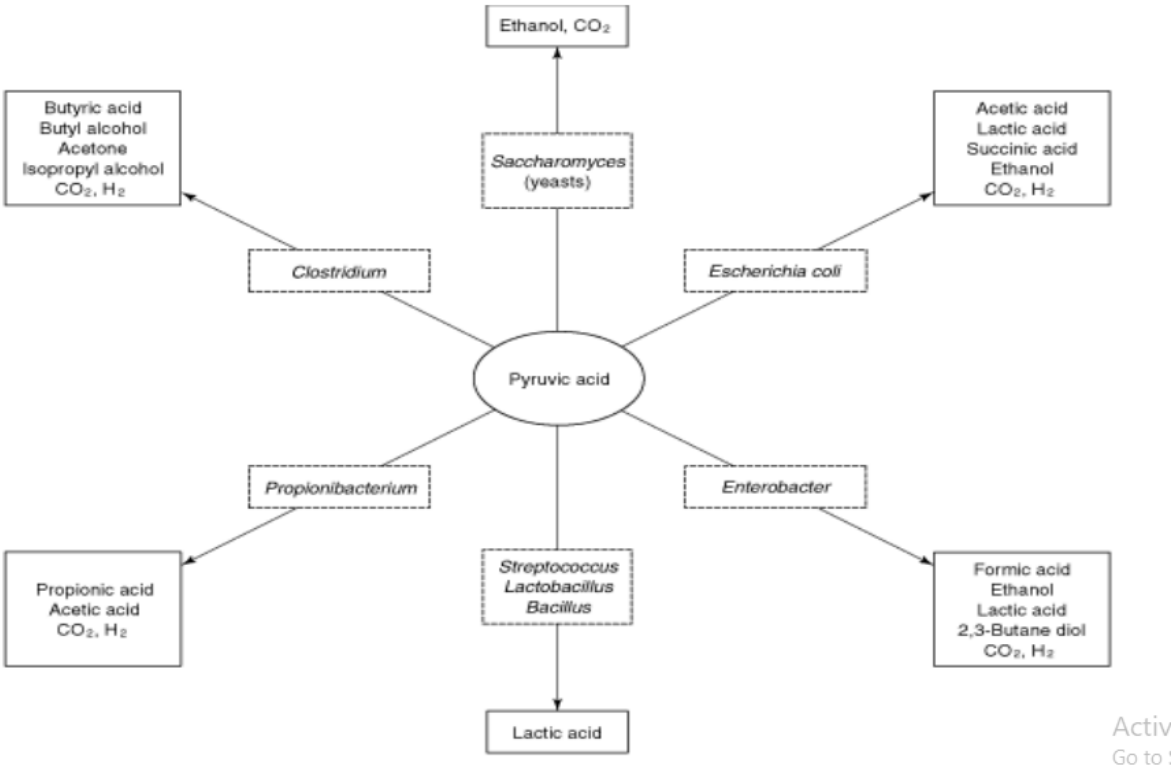
Catabolism: breaking down glycogen into glucose, releases energy (exergonic)



Overview of fermentation products formed from pyruvic acid by different bacteria:

Fermentation (usually in absence of O2) refers to the breakdown of a sugar (maltose or glucose) to pyruvic acid. This process is important for the identification of pathogens. Fermentation is also called the glycolytic (breakdown) cycle, and this process by is done by facultative bacteria to generate ATP in the absence of oxygen. If oxygen is present, the pyruvate enters the Krebs cycle and is metabolized to two final products (water + CO_2). Krebs cycle generates much more ATP than the glycolytic cycle; therefore, facultative bacteria grow faster in the presence of oxygen. Facultative and anaerobic bacteria ferment, but aerobes, which can grow only in the presence of oxygen, do not.

Aerobes, such as Pseudomonas aeruginosa, produce metabolites that enter the Krebs cycle by processes other than fermentation, such as the deamination of amino acids. During fermentation, acidic end products (pyruvate and lactate) are generated, which can be detected by an indicator that changes color upon changes in pH. For example, if a sugar is fermented in the presence of phenol red (an indicator), the pH becomes acidic and the medium turns yellow. (E-coli + Shigella we use lactose to be fermented in order to distinguish between them)



Iron metabolism:

• Ferric ion is required for growth of bacterial cell (essential component)

• Bacteria produce iron-binding compounds (siderophores)

Iron binding compounds take ferric iron from host cells.

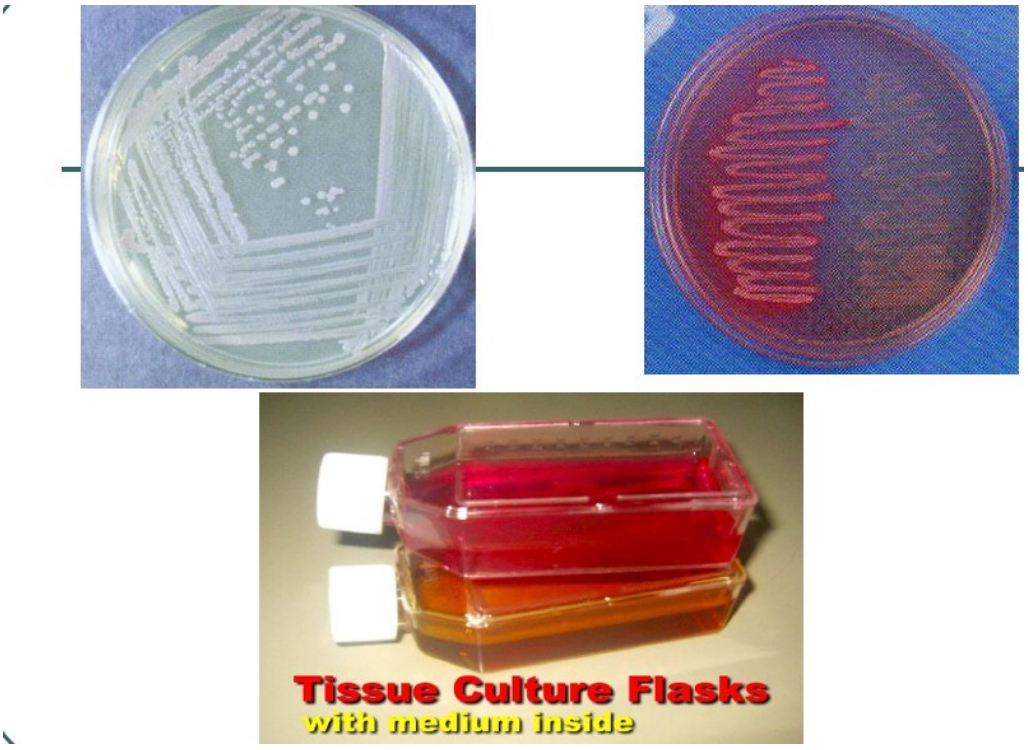
- **Culture media:**

Culture: is the most commonly used method for growth and identification of the infecting agent in vitro.
Several methods for diagnosing bacterial or fungal infections require the suspected pathogen to be isolated in a pure culture from a properly obtained clinical specimen (here I can do research and studies on it). This is accomplished by using an agar-based medium (very nutritious isolated colonies- every colony is one cell and a pure culture). After agar-based medium and obtaining a pure culture, I then stain it, and proceed to do biochemical testing.

- ✓ In the respiratory system- throat culture
- ✓ In the GI tract – stool culture (simulated stool culture).
- ✓ In the Urine tract –Urine culture.

*** We must know that there is normal flora in them.

As soon the specimen arrives the lab, urine specimens should at maximum spend one hour (from voiding to lab) and if we want to store it we can do so for only 18 hours and after this time it won't work efficiently and effectively.



- ✓ **There are two parts to this test:**

*Quantitative (how much bacteria is in the specimen)

*Qualitative (should only find one type of organism)- in women there is urine contamination + figure contamination of normal flora which cross tracts and cause infection.

If there is an ill person who is complaining from pharyngitis or another disease, **which specimen will we take that will reveal whether it is a viral or bacterial infection?

I should take a swab of the pharyngitis (without blood cells if it is localized infection).

** If it was a systematic infection, we can take specimen from a blood sample or others.

**** Which type of specimen will we take?**

According to the type of disease, such as:

1- **Pyelonephritis** (inflammation of the kidney, typically due to a bacterial infection)
2- **Cystitis tissue** (نسيج التهاب المثانة)

Here in both examples we will decide to take urine specimen.

- **Specimen:** The primary connection between the clinical encounter and diagnostic laboratory.
- Good quality of specimen is very important.
- Obtaining it properly to avoid contamination from the normal flora.

Bacteriology lab – we do microscopy after staining (gram stain is the most and it is considered to be a differential stain). يتم التفريق من خلالها بين البكتيريا

If it is gram negative, then it may be Neisseria meningitis.

And if it is gram positive –diplococci it may be Streptococcus pneumonia.

But if it is gram negative-rods it may be -H Influenza.

Gram stain is important for diagnosis

- If we have specimen that doesn't have normal flora (CSF/Blood/sputum specimen), after staining it, we can look at it in microscope and immediately decide what is the disease, such as concluding that there is meningitis after looking at a CSF specimen.

- Sputum differs from saliva

- Microscopy is important for determining the needed culture.

*PCR-replacing culture-DNA Identifying **–ex**:

- **Tuberculosis** (**TB**) in an agar plate it takes 6 weeks to start to be noticeable.

- **Chlamydia** (obligate intracellular parasite)

Because they are hard to grow and require tissue culture labs, long duration and expensive; we go directly to identifying DNA.

- Legionella Pneumonia (causes a dangerous disease) **Haemophilus Pneumonia**, infect through inhalation, exist more in hospital water, difficult to culture, grow and isolate it, so we use identifying DNA.

** When we obtain an organism and in the lab determine its type we can then decide what the suitable antibiotic –prescription is.

Sometimes we can grow bacteria:

- Chlamydia, we go to immunology.
- Viral infection (in tissue culture-takes long time-), we go to serologic (using serum) –it means to look for the presence of antigen or antibody in specimen, this method is easier, for example; Mycoplasma.

*Mycoplasma is very small, wall-less, and takes time to make colonies; therefore it is easier to use the serologic method.

- Serum= plasma-blocking factors (all proteins without microorganisms).

- Plasma contains anticoagulation, it is the largest part of your blood. The proteins and antibodies in plasma are also used in therapies for rare chronic conditions. These include autoimmune disorders and hemophilia.

* When separating blood there are white blood cells which are called the buffy coat.

- Swab must be sterile (free from organisms)

- If I take a specimen from an affected person of Neisseria Meningitides, the specimen is fragile (which means that it can't tolerate temperature or acidity of medium or others). So we need transport media –in a tube or other- to transport it to a lab. Transport media are fluid/semisolid agar + free from O_2 + neutral PH, which will minimize bacterial growth such as normal flora. Transport media relate with the buffy coat.

- ## Types of Samples:

1- ***Direct sample***: could be fluid (like CSF) or from tissue -which is difficult to obtain-like specimen from liver or lung...

2- ***Indirect sample***: It is invasive, easier, convenient for both patient and physician, need inspiration ex: (voided urine + expectorated sputum).

*** Microscopic Examination: before we want to see bacteria, it has to be stained.

******* TYPES Of culture media:***

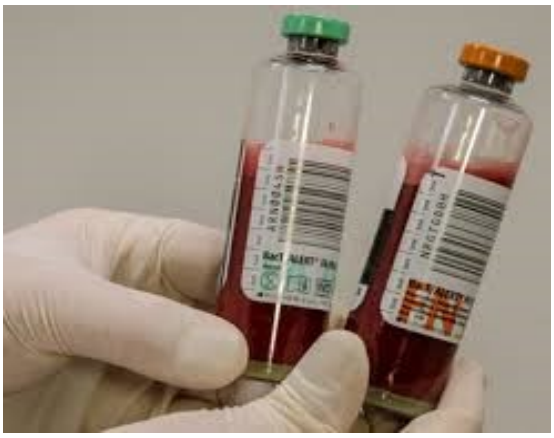
1- For culture you can use liquid medium (mix not pure), put the sample in nutrient growth (takes a day's duration), so we can observe growth, for example; CSF/Urine specimen which is clear (normally), doesn't have turbidity –if it has we know that there is a disease, in this sample it is very easy to realize if there is something wrong or not (infection if it exists, the sample will contain WBC/Bacteria.)

2- Solid medium is better than the previous, in this procedure we can obtain an isolated colony-from one cell-, this called **pure culture**.
* Colonial morphology (characteristic feature of organism): dry or smooth-color: yellowish, shape: round.

- **E-coli** (gram negative)- flat, irregular, doesn't produce a capsule.
- **Klebsiella pneumoniae** (gram negative)- smooth, round, has a polysaccharide capsule.

****** Blood culture:***

is a laboratory test in which blood is injected into bottles with culture media to determine whether microorganisms have invaded the patient's bloodstream.



*__Sepsis__= blood poisoning= massive infection

*__Septicemia__ (bacteria in blood is multiplying): serious condition.

*__Bacteremia__: Bacteria is present in the blood, which is transported through the circulatory system to another organ, which in turn causes disease, (ex. Meningitis), if we want to take blood specimen we should take them 3 times within 24 hours.

*** If there is growth of bacteria => there will be turbidity, which we will then stain it in gram stain, identify the disease, and choose a suitable antibiotic for it.

***Throat culture** is commonly used to detect the presence of a Beta-hemolytic streptococci (cause pharyngitis). They are also used when there is thrush (candida).

* **Wound culture**- capsular swelling test (antibody against the capsule), we add bacteria that is isolated + add a drop of the reagent (antibody + capsule = enlargement/swelling), ex. S. aureus (gram positive cocci).

In the future we will depend almost fully on **PCR**, which will reduce time wasted and is highly specific and sensitive.
We get the specimen in the tube then we add primase +nucleotide +DNA polymerase and put it in the thermal circular and we program it (in the final slide look at the temperatures + PCR is explained in molecular biology as well).