**\*Microbiology:-It is the study of organisms that are too small to be seen with the naked eye .**

**These organisms are unicellular or multicellular ;they include prokaryotes(such as bacteria) & eukaryotes(such as fungi& protists) . viruses are also studied.**

**-Causative agents of the most common diseases are m.o which cannot be seen without using the microscope, therefore it is an important apparatus in the laboratory.**

**\*\*Microscopic Parts:- In order to operate a microscope properly & effectively, it is necessary to have an understanding of some of the various parts of the microscope & their functions.**

**1)Base: It supports the microscope &give the apparatus stability.**

**2)Arm: the part of the microscope that you carry the mic roscope with.**

**3)Body tube: The long tube that holds the eyepiece & connects it to the objectives.**

**4)Ocular lenses(eyepieces):The lenses in the upper part of the microscope where you look through to see the image of your specimen.**

**There are 2 kinds of microscopes :-**

**a)Monocular microscopes: have one ocular lenses.**

**b)Binocular microscopes :have two oculars.**

**5)Revolving nose piece : The rotating part of the microscope at the bottom of the body tube ,it holds the objectives.**

**6)Objective lenses : The microscope may have 2,3 or more objective attached to the nose piece ,they vary in length (the shortest is the lowest power or magnification, the longest is the highest power or magnification).**

**A-Scanning lenses (4X)**

**B-Low power lenses(10X)**

**C-High power lenses(40X)**

**D-Oil immersion lenses(100X)**

**7) Coarse adjustment knob :- The large round knob on the side of the microscope used for focusing the specimen, it may move the stage up or down to correct distance from objective for viewing.**

**8)Fine adjustment knob : The small round on the side of the microscope used for focusing the specimen after using the coarse adjustment knob.**

**9)Stage: the large flat area under the objective ,it has a hole in it that allows light to pass through. The slide is placed on the stage for viewing.**

**10)Stage clips: The clips on the top of the stage which hold the slide in place.**

**11) Aperture :the hole in the stage that allows light to pass through for better viewing of the specimen.**

**12)Diaphragm & condenser: the parts of microscope that control the amount of light going through the apertures.**

**13)Light: the source of light usually found near the base of the microscope , the light source makes the specimen easier to see.**

**\*Oil immersion lens: it is one of the objective which has the highest magnification ,it is used for the examination of the organisms that could not be seen without high magnification . a special oil is used with this objective which is called ((cedar oil)).**

**\*the function of oil:-the oil contributes to 2 characteristics of the image viewed through the microscope:-**

* **Finer resolution.**
* **Finer brightness.**

**These characteristics are important when high magnification is used ,so it is only the higher power objective that is usually designed for oil immersion lens.**

 **\*the reason of using oil: when the light passes from a material of one refractive index to material of another ,as from glass to air or from air to glass ,it bends.**

**Placing a drop of oil with the same refractive index as glass between the slide & the objective lens eliminates 2 refractive surface &increase the clarity of the image.**

* **Cleaning the oil immersion lens:**

**a disadvantage of oil immersion viewing is that the oil must stay in contact with the glass & oil is viscous.**

**Oil immersion lenses used only with oil ,& oil cann’t be used with dry lenses ,such as:- low & high power lenses , therefore oil distorts image seen with dry lenses - so once you place oil on a slide ,it must be cleaned off thoroughly before using the high dry lens again. Oil on non –oil lenses will distort viewing & possibly damage the coatings.**

**Cleaning this lens is done by using:-**

**1-Dry “lens paper”**

**2-Wet lens paper with “xylole”**

**3-Dry lens paper.**

**\*Calculating the total magnification:-**

 **Objective lens magnification \* Ocular lens magnification.**

 **Example: 40X\*10X=400X**

**-----------------------------------------------------------------**

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**Laboratory Equipments & Apparatus**

**-test tube -lens paper -needle**

**-plane tube -triple holder -funnel**

**-universal tube -flask -pipette**

**-centrifugal tube -petridish -spreader**

**-benzen burner -rack -Pasteur pipette**

**-washing bottle -loop -slide**

**-filter paper -cylinder -cover slip**

**-swab -beaker (slide cover)**

**1\*Autoclave**

**-components:-**

1) jacket 2)chamber 3)door or cover 4)door handle

5)thermometer 6)pressure gauge 7)excessive water discharge

8)air discharge valve 9) safety valve valve

**-Uses:-**

1)sterilization of cultural media & lab. Equipments.

2)treatments of contaminated solutions & fluids.

**Principle of work:-**

1) heating water in a closed vessel which leads to the production of saturated steam under pressure, its temperature is more than 100Cْ & this is called “ moist heat sterilization”In presence of pressure.

2)sterilization circumstances are ( 121) C for (15) min under pressure of ( 1) atmosphere ( which equal to 15 pound/inch2).

-working steps:-

1)fill the jacket with water to the level of holes & make sure that it does not reach the chamber.

2)put the chamber that contains materials & equipments to be sterilized in the jacket.

3)close the door by closing the door handle without doing that tightly.

4)open the air discharge valve.

5) switch on the electrical current for heating water inside the apparatus.

6)wait for 3-4 minutes until acontinuous homogenized flow of steam discharging valve indicating the elimination of all trapped (enclosed) air in the apparatus.

7)close the air discharging valve & tighten the door handle ,then monitor (watch) the temperature & pressure guage for reaching the required degrees ( 121C & 1 atmosphere respectively),so we calculate time at this point.

8-switch off the electrical current when time over( 15 minutes).

9- wait until temperature decreases under 100C ,then open air discharging valve for reaching equilibrium in pressure inside & outside the apparatus.

10-when the whistling voice (which indicate steam discharging) stops ,open the door handles then open the door & wait until the contents of the autoclave become cool, then raise the chamber which contains the sterilized materials& dry them from water drops formed on it.

**\*Importance of air elimination (air removal):-**

**The impotance of the enclosed air removal inside the apparatus (when the operation starts) belongs to the necessity of exposing materials & equipments which we want to sterilize to the effect of steam only because:-**

**1)mixing air with steam lowers the temperature that is necessary for the required pressure.**

**2) as air density is more than of steam, it will form a separated cold layer at the lower part of the apparatus ,**

**This will delay the penetration of steam to the materials and equipment to be sterilized this preventing enough heating.**

**2\*Electrical oven**

**-components :- 1)** thermostat 2) thermometer 3)timer .

**-uses:-**sterilization of the lab. Equipments wither glass or metallic.

**-principle of work:-**

1)sterilization by using dry heat.

2)sterilization circumstance are ( 160-180) C for ( 1-2) hours.

- working steps:-

1)put the equipments to be sterilized in the apparatus (make sure of its dryness).

2)fix the thermostat at the required degree & switch on the apparatus.

3) watch the thermometer which raises gradually .

Calculation the time begins upon reaching the required heat.

4)switch off the apparatus when time over & wait until the heat decreases to (40) C , Then open the door of the oven & take out the sterilized equipments.

**3\*Incubator**

**-components :-**

1) chamber of different size contains glass or metallic shelves

With two doors: outer metallic door & inner glass one.

2) thermostat 3) thermometer

**USES:-**

Incubation & encouragement of the growth of all M.O.kinds (types) like pathological & non –pathological bacteria ,parasites & fungi.

**Pathological bacteria ------------< 37C**

**Fungi------------------------- <25 – 28C.**

**4\*Centrifuge**

**-components:-**

1)central head

2)wind shield or rotary piece fixed on the central head & branched into several branches called (pans) which carry a(bucket) at its end or number of buckets specialized to carry one tube or several tubes which contain the fluid to be centrifuged .

3)timer which stops the centrifuge spontaneously when time is over.

4) revolutions calculator which refers to the velocity of the apparatus during centrifugation (round per minute/revolution rpm).

**Uses:-**

**Separations of fluids or suspended culture of M.O into two parts:-**

**1-sediment which is the lower part that is located at the bottom of the tube that contains the suspended particles in the fluid.**

**2- supernatant which is the upper part that lies above the sediment & contains the rest of the fluid.**

**-principle of work:-**

Rotating the central head in a circular movement that lead to rotation of the tubes which contains the fluid to be centrifuged,

So it becomes subjected to a force that attracts it away from the center, called “centrifugal force “ & then fluid will be separated into sediment & supernatant.

**-working steps:-**

1)switch on the apparatus & elevate (increase)the velocity (speed) gradually until reaching the required velocity.

2)fix the timer at the required time & when time over ,

The velocity of the centrifuge will decrease gradually then take out the tubes slowly after opening the cover.

**5\*hood or inoculation cabinet**

**-component:-**

1)small chamber closed from all sides by windows made of glass with small front hole for entering hands in order to manipulate the laboratory work.

2)ventilation hole contains filters & found at the roof of the chamber for sucking air which contains microbes or for draining

Gas burning residues which formed because of using benzene burner during work.

3)ultra violet ray light.

4)electrical light at the chamber roof for lightening the chamber.

**Uses:-**

**Performing laboratory culture in a sterilized circumstance away from air currents to prevent contamination .**

**6\*hot plate with magnetic stirrer**

**-components**

1)electrical motor

2)base for putting container( in which the fluid mixing done) on it.

3) heating gauge.

4)speed gauge.

**Uses:-**

Dissolving culture media powder in distilled water for preparation of that medium in order to convert it into homogenized solution.

To make that easy we use small magnetic bar which must be put in the medium to facilitated the homogenisity.

**7\*water bath**

It is used for dissolving solid culture medium after its solidification in order to convert into liquefied or melted agar to facilitate pouring it into plates or test tubes.

**8\*colony counter**

It is used for counting single colonies which are grown on the surface of solid medium after using some culturing isolation methods.

**9\*vortex**

It is used for converting the bacterial suspensions & other suspensions into homogenized solutions especially those that are cultured in test tubes.

**10\*balance**

It is used for weighing large quantities of materials or powders of cultural media.

**11\*sensitive balance**

It is used for weighing small quantities or few grams of materials or powders of cultural media.

Sterilization

\***Sterilization :-** a physical or chemical process that completely destroy or removes all microbial life, including spores.

\***Disinfection:-** a physical or chemical process that kill or prevent the growth of pathogenic microorganism but not necessarily the spores.

\***Sterilization methods:-** there are many methods for sterilization , in general they are divided into two methods:-

**A- physical methods B- chemical methods**

 **A-physical methods:**

1/ heat

2/ filteration

3/ radiation

 1.1 Dry heat

1/ **Heat :**

 1.2 Moist heat

**1.1. Dry heat**

**1.1.1 red heat :** in this method , the tools (especially the metallic ones) are exposed to the flame of benzene burner until reaching the red color. These tools include :-

1\* loops 2\*inoculation needles 3\* forceps

**NOTE**

the tools (which are sterilized by using the red heat method)

 are made of steel that resist oxidation & high temperature.

**1.1.2.flaming :** in this method, the tools are exposed to the flame of benzene burner without allowing them to reach the redness point the tools include:-

1)the mouth of the test tubs & flasks.

2) slides . 3)cover slips(cover slides).

**NOTE**

For more precision ,alcohol can be used by immersing the above mentioned tools in it before the exposure to the flame.

e.g. knives used in the surgery (dissection scalpel) & medical syringes& spreaders.

**1.1.3. Hot air oven or electrical oven:** in this method, the tools are sterilized at(160-180)C0 for (1-2) hrs.it is a perfect method for glass wares sterilization . the glass wares include: 1-tubes 2-flasks 3- petridishes

4- pipettes.

This method is suitable for sterilization of metallic tools that ar not affected by high temp.

 E.g. knives (dissection scalpel) & forceps.

**NOTE**

 :moist glass wares must be dried before sterilization by using

this method to prevent smashing (breaking).

**1.2.Moist heat**

**1.2.1. pasteurization :** in this method, sterilization is done by using temp.

Less than (100) C0  to destroy pathogenic bacteria by heating at (62) C0

For (30) mints (LTLT) or (72)C0 for ( 15) secs.(HTST) .

The materials which are sterilized by this method are:-

1) serum. 2) body liquids (which contain albumin). 3) milk.

**1.2.2. Boiling :-** in this method , sterilization is done by using (100)C0 for (10) mins.

It is enough to kill all the pathogenic m.o. in vegetative phase(but not their spores).

This method can be used if there is no alternative method of sterilization. It is used for sterilization of materials & instruments(tools)(wither metallic or glass).

**1.2.3. tyndallization or steaming:-** in this method , sterilization is done by exposing the materials to the vapour of boiling water for a couple of minutes & for (3) days in sequence.

The materials which are sterilized by this method are :1) suger solutions.

2)enzymes. 3) vitamins 4) antibiotics.

**NOTE**

 : this method is used for the sterilization of the material which may be destroyed by using the high temperature

Note

 :- this method is not efficient to kill the spores that grow at high temperature.

Note

:- the sterilization in this method is done for 3 days in sequence

because:-

1 st day ------------< the vegetative cells are killed.

2 nd day -----------< the spores convert into vegetative cells.

3 rd day -----------< the converted vegetative cells are killed,in addition the sterilization process is completed effectively.

**1.2.4.Autoclave :** in this method , sterilization is done by using temp.

Higher than (100) C0 (steam + pressure).

The materials & instruments (glass or plastic) which are sterilized by this method are:

1\* cultural media. 2\* solutions & liquids. 3\* cottons. 4\* other materials which are usually destroyed by using dry heat sterilization (oven).

NOTE

the reason of using autoclave (moist heat) for the sterilization of cultural media & other liquids is the existence of the steam which prevent the vaporization of the media & other watery solutions by heating ,but if these media & solutions are sterilized by an oven (dry heat) , they will be decreased in volume because of the oven environment that leads to the vaporization of these solutions.

note

 :- the sterilization by using an autoclave kill all kinds of pathogenic m.o & their spores (which can not be killed by other sterilization methods).

**2 // filteration** :- this method involves filtering th solutions or any other liquids through special sterile filters.

These filters allow the liquid to penetrate while the m.o are trapped on the surface of the filter.

The materials wich are sterilized are:-

1) serum. 2) enzymes. 3)vitamins. 4) antibiotics . 5) sugar solutions.

NOTE

 The most important filters are cellulose membrane filters which are made of cellulose nitrate or cellulose acetate e.g Millipore filters. This kind of filters is common because: **1-**High speed of filteration.

**2-**The membrane filter disc can be directly removed and placed on the surface of an appropriate (suitable) solid medium in order to make the microorganism grow on that medium, so the growth characteristics can be studied.

**3/// Radiation:** The ability of the sunlight to kill the m.o. is due to the ultra violet (u.v.) ray existence in it. The rays can be devided in it. The rays can be devided in to:

**1) Non –** **ionizing ray.**

**2) ionizing ray.**

**1-Non-ionizing ray:** e.g. u.v. light which is effective in the wavelength about (240-280) nm.

**\*Uses:** Sterilization of (1)hospitals (2)laboratories (3)meat package factories.

 Note

 The Sterilization by using u.v.l. is more effective on a clean surface because its inability to penetrate more than some millimeters in liquids and solid surface, so it is used only to sterilize clean surfaces.

**2-Ionizing ray:** e.g. Gamma ray

**\*Uses**: Sterilization of (1)food products (2)penicillin (3)plastic items(which can be consumed).

**B-Chemical methods:**

Those methods are used for the Sterilization of rooms and floor. The efficiency of these methods depends on:

1-Chemical agent concentration.

2-Time period required for Sterilization.

3-Quantity and quality of m.o.

**\*Disinfection or antiseptic**: It is a chemical agent or materials which have the ability to kill or prevent growth or metabolism of m.o. vegetative cells but its efficiency on their spores is not confi

**\* Disinfectants**

1-**Volatile Dis** → chloroform

**\*Uses:** serum which is added to the cultural media.

Note

 Chloroform can be used in the form of liquid or concentrated vapor state by exposing the liquid or contaminated surface to it for about (1)min.

2-**Phenols** → Lysol, Cresol, Hycoline, Phenol

**\*Uses:** (1) surgical tools (2) bacterial cultures (3) serum (4) vaccines.

3-**Metalic salts** or heavey organic compounds of metals→ mercuric chloride

4-**Gases**→ formaldehyde

**\*advantages:** (1) not expensive (2) water soluble (3) effective for killing all kinds of m.o. and their spores (4) have no damaging effect to the surfaces to be disinfected.

**\*disadvantages:** This gas causes irritation of the respiratory tract when it is inhaled.

**\*Uses:** (1) rooms and fur natures (2) laboratory environment (3) laboratory cultures (formalin)

Note

 This gas (formaldehyde) is also used in a liquid state (formalin) which disinfects by direct contact with the contaminated surfaces.

Note

 The gas (formaldehyde) is used for the disinfection of the materials that cannot be exposed to the liquid solutions which may damage those materials.

(5) **Halogenes**  chlore \*Uses: water disinfection

 Iodine \*Uses: wounds dis.

(6) **Alcohol**→ Ethanol with the concentration of (70-75)% ,This concentration causes melting (lysis) and damaging of the lipid membranes and denaturation of the microbial cell proteins.

Note

 Alcohol is used in conc. Of 70% rather than 90% because the aqueous solution (diluted sol. ) of alcohol is more effective in disinfection than the absolute alcohole, and this is due to the m.o. cell wall proteins which are more soluble and easily damaged when the diluted alcohol is used.

(7) **Detergents**: They are effective on some kinds of bacteria.

\*Typical properties of disinfectants:

(1)Non-toxic to human.

(2)Not lossing its ability of disinfection after it is diluted.

(3) Have no damaging effect on the surface of the material to be disinfected and its efficiency acts only with m.o.

**Culture media**

By knowing environmental and nutritional factors that affect the growth of specific prokaryotes, it is often possible to provide the appropriate conditions for their cultivation.

**\*Culture media:** it is the environment where the microorganism lives and gets its own nutrients from nutritional materials forming that environment in order to grow & reproduce in laboratory .

**\*Components of the Typical Culture Medium:**

 2. Nitrogen source . 1. Carbon source. 3. Phosphate source. 4. Water source . 5. Source of different minerals e.g. iron, magnesium, sodium, potassium and traces of zinc and manganese.

 **Note:** Some m.o. may need a source of vitamins and amino acids which are important in building cellular components of m.o.

**\*Culture Media Importance:**

 1) Isolation and preservation of m.o

 2) Reproducing a m.o and studying its characteristics.

 3) Encouragement and induction of the m.o to produce materials of industrial importance like production of antibiotics and some organic acids.

**\*Division of Culture Media :**

 **A// Depending on its consistency:**

 1) Liquid media or broth: these are media that do not contain any percentage of agar.

They are usually used in the extraction of active compounds produced by m.o. e.g toxins.

 2) Solid media: these are media that contain (1.5 - 2) %. agar. They are used for the isolation of m.o in the form of pure colonies and for isolation of two kinds (or more) of bacteria.

 3) Semisolid media: these are media that contain less than 1% of agar, about(0.7 – 0.8) %.

This amount of agar is added to the liquid medium so it becomes gelatinous. These media are used for studying of the bacterial motility and their demands for O2 in order to know if these m.o are aerobic , anaerobic , micro aerobic , or facultative anaerobic

 **Note:** In order to obtain solid culture media , we use the following materials:-

 1)) Gelatin:

 1- It is added to the medium in a percentage of (5-10) %.

2- Its usage is limited because it liquefies at incubation temperature (37)ºC and solidifies at (25)ºC. Further, some kinds of bacteria can utilize it and dissociate it because they have "gelatinase" enzyme .

 2)) Agar:

 1- A complex carbohydrate material extracted from the red algae.

2- It is considered a typical solidifying material because it doesn't have nutritional value for the bacteria so it isn’t attached or utilized by the bacteria. In addition it liquefies at (100) ºC and solidifies at (40- 45) º.

 **B// Depending on its nature or components or contents:**

 1) **Natural media**: these are media that contain natural materials e.g. plant or animal tissues , milk , diluted blood , fruit and vegetables juice ( like tomato and potato) and meat extract . The components of these media are accurately unknown e.g. tissue culture which is prepared from chicken embryo and used for cultivation of viruses.

 2) **Artificial media:** these are divided into:

A) ***Synthetic or simple or defined media:*** these are media which contain chemical substances that we know their composition and concentration accurately. These media are used for studying:

 1- The nutritional requirements of m.o .

2- The effect of each of the substances forming these media on m.o .

 b) ***Semi synthetic or complex media:*** these are synthetic media supplemented with natural components of unknown chemical composition like the addition of meat extract, yeast extract, pepton or serum.

These media are used when the studied bacteria are sensitive to synthetic media so they are cultured or grown on media supported with natural sources.

 **Note:** Synthetic media are used for cultivation of fastidious bacteria while semi synthetic media are used for cultivation of non-fastidious bacteria.

3) Living media: these are media in which living cells are used as culture media like the use of chicken embryo for cultivation of viruses.

 **C//Depending on the purpose of uses:**

 **1) General purpose media:** these are media in which many m.o are grown .They are used for many purposes e.g. nutrient broth.

**2) Selective media:** these are media that are used for the cultivation and isolation of certain species of m.o from a mixture of different species .

**These media are divided into two kinds :**

 **2.A) Depressive selective media:** these are media which are used for the selection of the certain species of m.o by depressing the undesirable (un wanted ) species .

**There are several ways of depression like:**

 1- **Addition of some depressive materials to the medium** like :

 a- The addition of certain dyes or stains e.g. crystal violet, methylene blue, basic fuchsine which inhibit the growth of G+ve bacteria without affecting the G-ve growth .

 b- The addition of certain antibiotics e.g. cycloheximide which inhibits the growth of saprophytic fungi and allows the growth of medical fungi when it is added to sabouraud agar .

 2- **Using certain growth conditions and changing them according to the growth conditions of the desirable species** e.g. temperature , ventilation, and pH.

 **2. B) Enrichment selective media:** these are media which are used for the selection of the desirable species of m.o. by induction the growth of these species in a better way

 than the other species which are grown in the same medium and that is done by adding stimulatory materials which enrich the media like the addition of blood to the

 nutrient agar medium in order to form blood agar medium. These media are used for the cultivation of fastidious bacteria.

 **3) Differential or identification media:** these are media which differentiate between two different groups of m.o. and allow the diagnosis of m.o.

 depending on its biological characters (it means that these media contain certain material allows the detection of certain m.o.depending on a metabolic actiondone by that m.o.) e.g.

**MacConkey agar** which differentiate between lactose fermented bacteria and non-lactose fermented. The colonies of lactose fermented bacteria appear pink while the colonies of non-lactose fermented bacteria appear colorless (it means that these colonies have a color which is similar to the culture media color).

\*Lactose fermented bacteria ---------< pink colonies: E . coli

\*Non-lactose fermented b. --------> pale or colorless colonies: Neisseria , Pseudomonas, Proteus

\*pH - indicator----------> neutral red

\*Sugar --------> lactose

**Note**: - MacConkey agar is considered a depressive selective medium

 it permits the growth of G-ve enteric bacteria and inhibits the growth of G+ve non-enteric bacteria.

 **Why?** This medium contains (crystal violet) which is a dye that inhibits G+ve bacteria,

 and the medium is also contains (bile salts) which inhibit non-enteric bacteria and both of them (crystal violet and bile salts) do not affect the growth of enteric G-ve bacteria because this bacteria is adaptable to live with the presence of bile salts in the intestine .

**Note:-** Reagents or indicators are added to the differential media to differentiate between different types of m.o. which are grown on these media. Usually these reagents are dyes which investigate the change of the medium acidity as a result of a metabolic action done by the m.o. and this change in acidity is manifested by noticing the color change of the reagent ( the dye ) which is added to the medium and these reagents are called (PH-indicators).

 **MacConkey agar** Differential m. ----> Neutral red

 Depressive selective m.

 Crystal violet bile salts

 G+ ve √

 G- ve X Enteric b. √

 Non- enteric b X

 **4) Maintenance media:** these media are used formaintenance and storage of m.o. for along period by adding materials in a certain ratio. These materials maintain the persistence and viability of m.o. for a longer time e.g. glycerol or tween-80 which leads to the slow growth of m.o. because fast growth is followed by fast death and that is not desirable. There is a special medium for the maintenance of each m.o.and that medium is maintained in the freezer e.g. nutrient broth, brain-heart infusion broth.

 **5) Transport medium:** these media that are cultivated with the sample temporarily in order to transport it from its isolation source (human, soil, water, ….etc)

to the laboratory for maintaining and keeping its viability and other characteristics e.g. stuart medium which is used for transporting Gonnorhoea bacteria (Gonococci) and glycerol saline medium which is used for transporting stool samples.

 **6) Assay media:** these media are used for performing a particular test (assay) like the medium that is used for performing antibiotic sensitivity test which is called Muller- Hinton ager.

 **7) Stimulatory media:** these are media that stimulate the production of certain materials or structures inside the m.o. cell like: toxins, pigments and endospores.

 **\*Preparing the Culture Medium:**

 **Note:** You must read the instructions found on the container of the culture medium before preparing it .

1. Weigh the required amount of the medium powder by using the balance and put it in a flask or other containers.
2. Add the required amount of distilled water to the flask .
3. Dissolve the medium powder in the distilled water by using hot plate with magnetic stirrer or by using the flame of benzene burner or without using heat .
4. Adjust the pH of the medium to the required value (+ 0.1 or + 0.2).
5. Distribute the medium in the tubes or flasks or any other containers according to necessity .
6. Sterilize the culture medium by autoclave
7. Cool the medium after sterilization and keep it in refrigerator at (4) ºC until using it.

**Note:**

 1- It is preferred not using containers that made of cupper when you prepare the medium, instead of these you can use containers made of glass which are heat resistant.

 2- It is preferred using the distilled water for instead of the tap water for preparation of medium.

 3- It is preferred to adjust or fix the pH of the medium before sterilizing it in autoclave.

 **Q) Why you adjust the pH of the medium before sterilization to the required value with the addition of 0.1 or 0.2 ?**

The pH of the medium is adjusted before sterilization, so that it will be not contaminated when using pH-paper or when adding solutions used for fixing pH after sterilization.

 On the other hand, the reasons of increasing the value to the mentioned degree is related to the fact that pH or hydrogen ion concentration is affected by heat,

it means that the concentration decreases when the

temperature increases by heating medium in the autoclave (sterilization),

therefore you must elevate (increase) the value before sterilization to reach the required value after sterilization.

 **Q) How do you adjust (fix) the medium pH ?**

 1. By using the pH - indicator paper.

 2. By using the pH- meter.

 \*Adjustment of the medium pH is done as follows:-

 1. By adding a couple of drops of NaOH in the concentration of 1N .

 2. By adding a couple of drops of HCl in the same concentration.

 **Note:** You must cool the medium to (60)° C before the adjustment of the PH because the medium temperature affect the efficiency of the procedure especially when using the pH-meter.