Microbiological culture media

By

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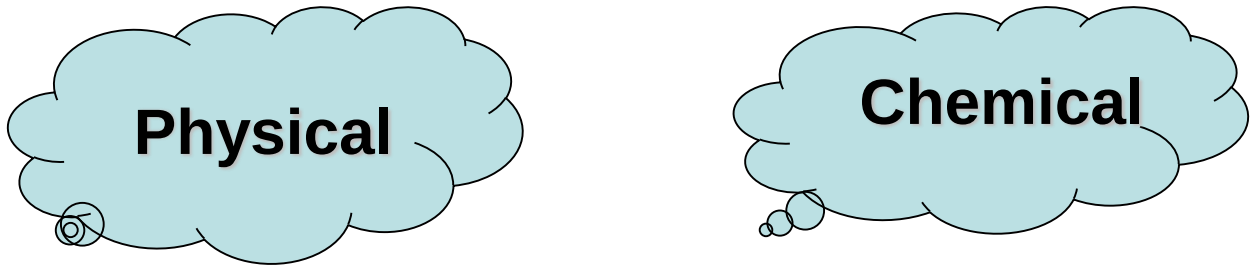
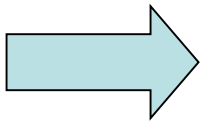
- **Microbial growth = increase in number of cells, not cell size**

The Requirements for Growth:



Physical Requirements



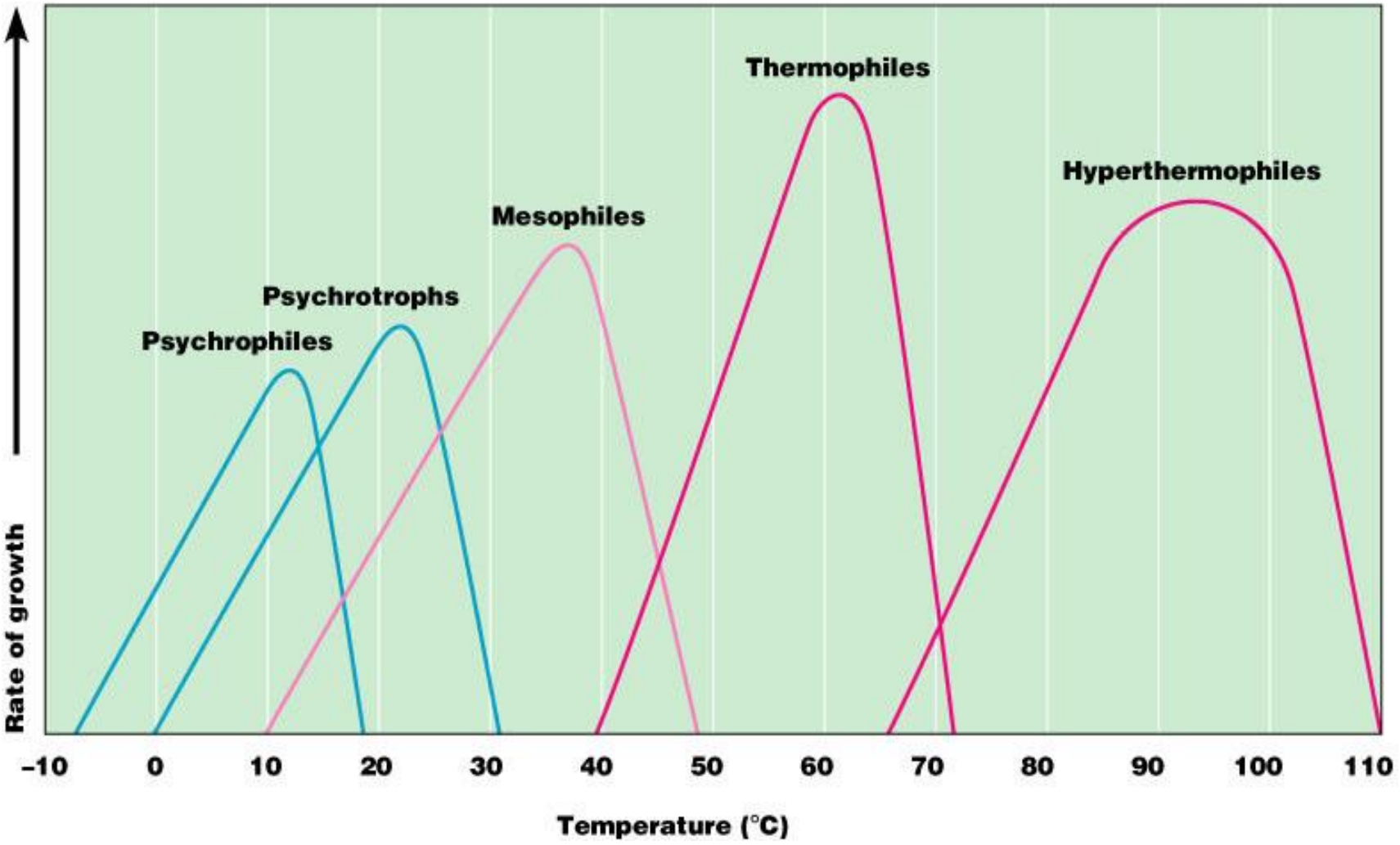
- Temperature
- PH
- Osmotic pressure

Temperature:

- Range min. to max.
- Minimum growth temperature
- Optimum growth temperature “ideal”
- Maximum growth temperature

Mesophiles 25 – 40 **o***C*

Thermophiles 50 – 60 **o***C*



Psychrophiles 0 – 20 **o**C
Optimum: 15 **o**C

Psychotrophs 0 – 40 **o**C
Optimum 20 – 30 **o**C

pH:

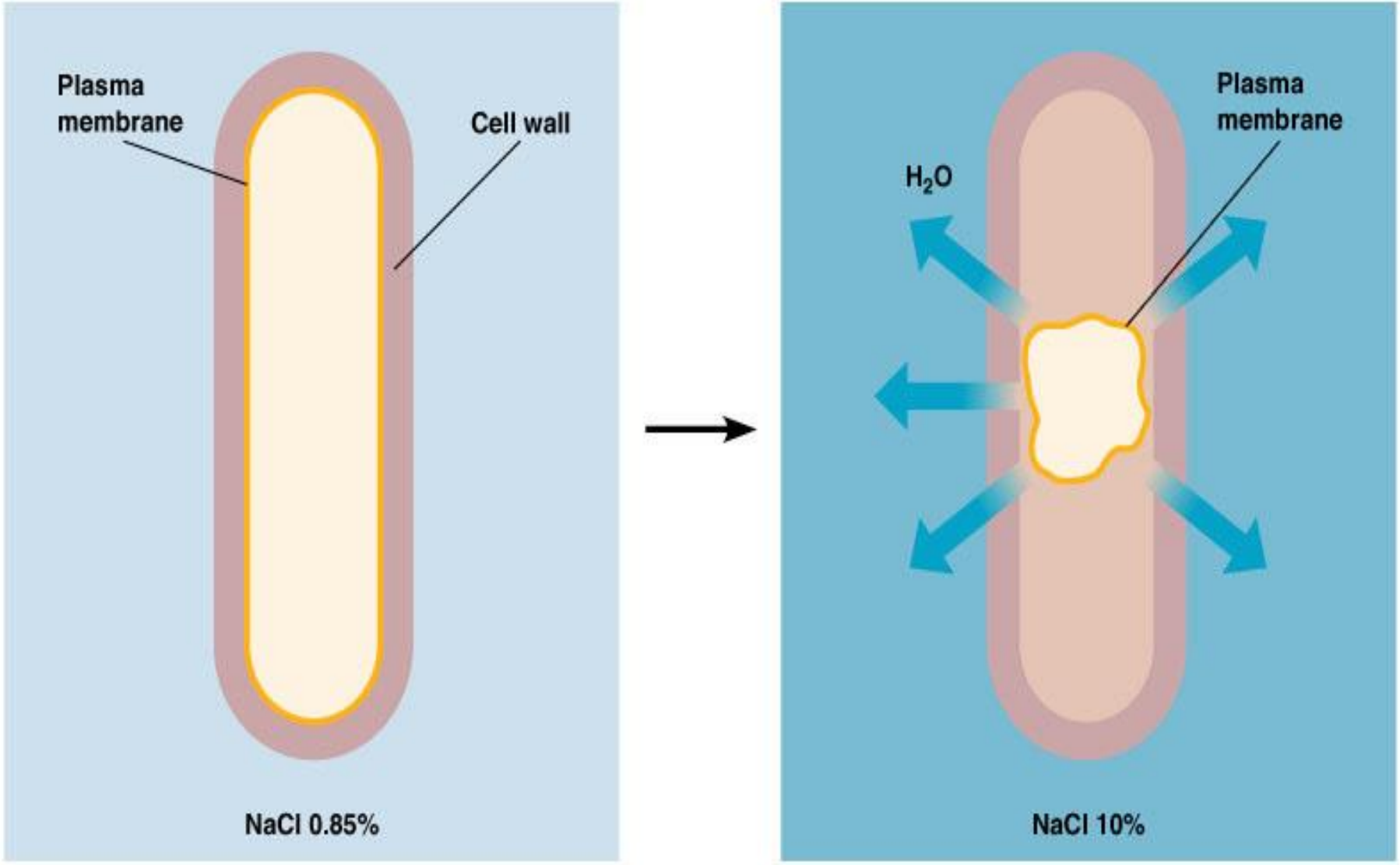
- This has to be monitored during the preparation of media since this will influence the kind of organisms that are able to grow in the medium.

- The pH of the medium will thus determine which organisms are able to grow on the medium.

- Most bacteria grow between pH 6.5 and 7.5
- Molds and yeasts grow between pH 5 and 6
- Acidophiles grow in acidic environments

Osmotic Pressure:

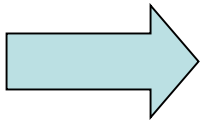
- **Hypertonic environments, increase salt or sugar, cause plasmolysis**
- **Extreme or obligate halophiles *require* high osmotic pressure**
- **Facultative halophiles *tolerate* high osmotic pressure**



(a) Normal cell in isotonic solution. Under these conditions, the osmotic pressure in the cell is equivalent to a solution concentration of 0.85% sodium chloride (NaCl).

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding than in the cell (hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

Chemical Requirements



- **Carbon**
- **Nitrogen**
- **Sulphur**
- **Phosphorus**
- **Trace elements**
- **Oxygen**
- **Organic growth factors**

Carbon

- Structural organic molecules, energy source
- Heterotrophs use organic carbon sources.e.g.glucose
- Autotrophs use CO_2

Nitrogen

- Inorganic source of nitrogen is ammonia (in the from of ammonium salts) for autotrophs or heterotrophs.
- Organic nitrogenous nutrients as amino acids or reduction of nitrates (in heterotrophs only).

Sulfur

- **In amino acids, thiamine, biotin**

are essential for the activity of enzymes.

Phosphorus

- **In DNA, RNA, ATP, and membranes**

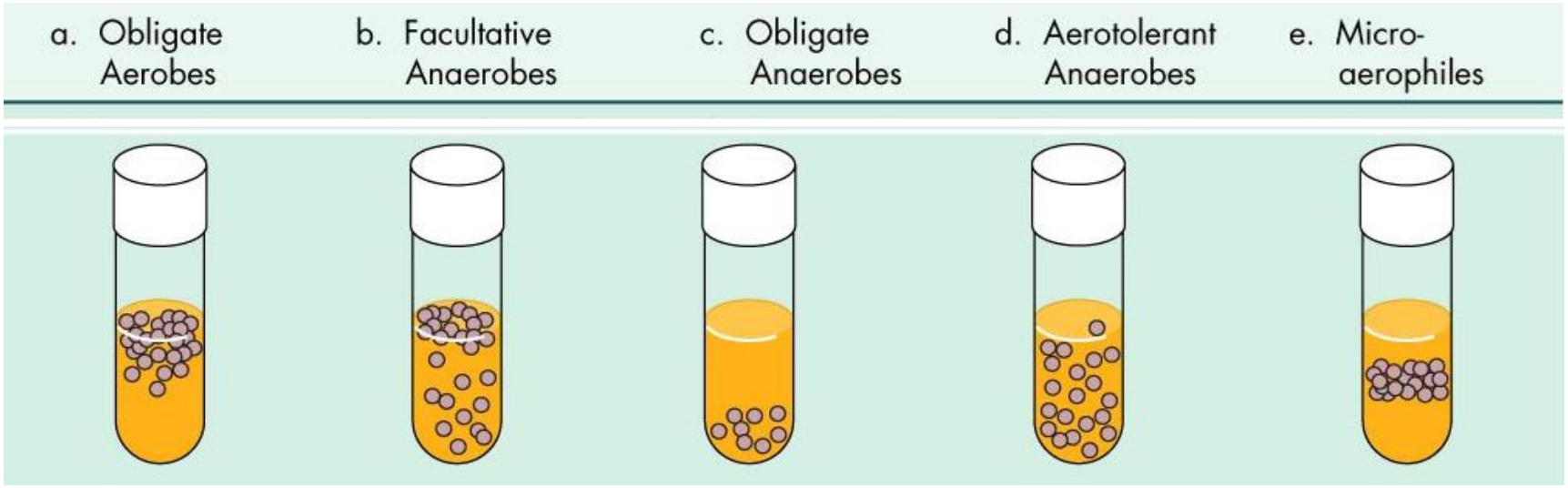
are essential for the activity of enzymes.

Trace Elements

- **Inorganic elements required in small amounts**
- **Usually as enzyme cofactors**
- **eg. copper, iron, magnesium**

Oxygen

- Some organisms need
- Other cannot live if O2 is present
- Some will tolerate



Organic Growth Factors

- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, pyrimidines

Bacteria multiply by binary fission

The population grows in geometric progression

1→2

2 →4

4 →8

8 →16

16 →32

32 →64

64 →128

128 →256

256 →518

518 →1036

1038 →2064

2064 →4128

4128 →8256

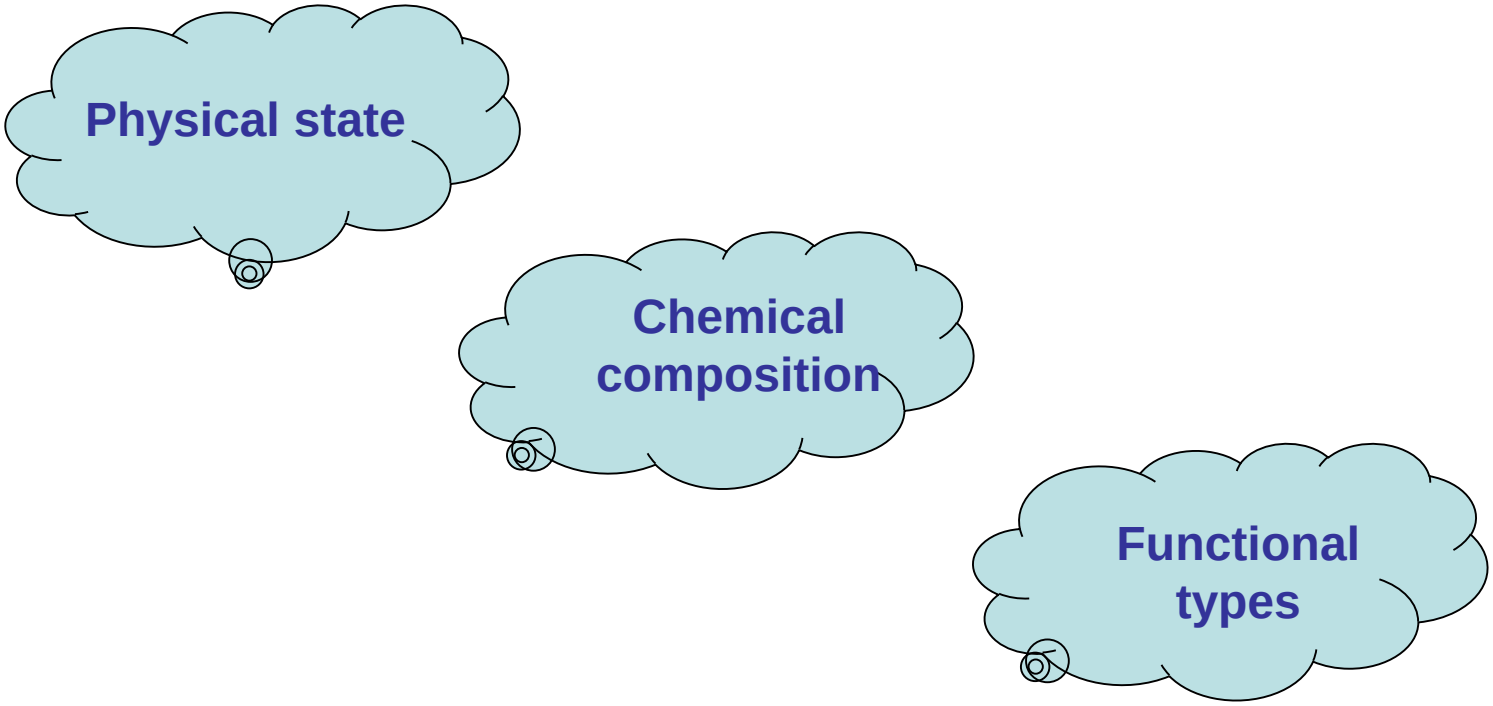
If 100 cells growing for 5 hours produced 1,720,320 cells:

The main purposes for cultivation of bacteria are:

- To grow and isolate all bacteria present in clinical specimens.
- To determine which of the bacteria that grow are most likely causing infection.
- To obtain sufficient growth of clinically relevant bacteria to allow identification and characterization.
- To prepare antigens, toxins and vaccines.

- **A culture medium** is any material prepared for the growth of bacteria in a laboratory
- Microbes that grow and multiply in or on a culture medium are known as a **culture**
- **Inoculum**: Introduction of microbes into medium
- **Pure culture** contains only one species or strain
- **Mixed culture** contains more than one species or strain
- **Fastidious bacteria** that require very strict nutrients.

Culture media are classified according to three properties:



Classification of culture media according to physical state:

Bacterial growth media can be divided into 3 main types, depending upon the physical state :

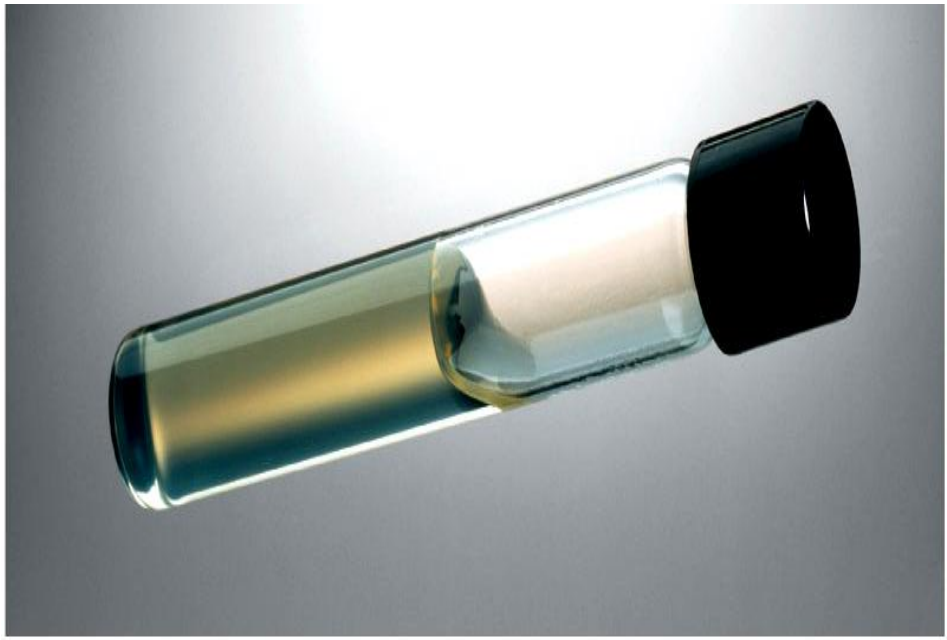
1. **Liquid media**
2. **Semi-solid media**
3. **Solid media**

Liquid media are water-based solutions that are generally termed broths, milks and infusions.

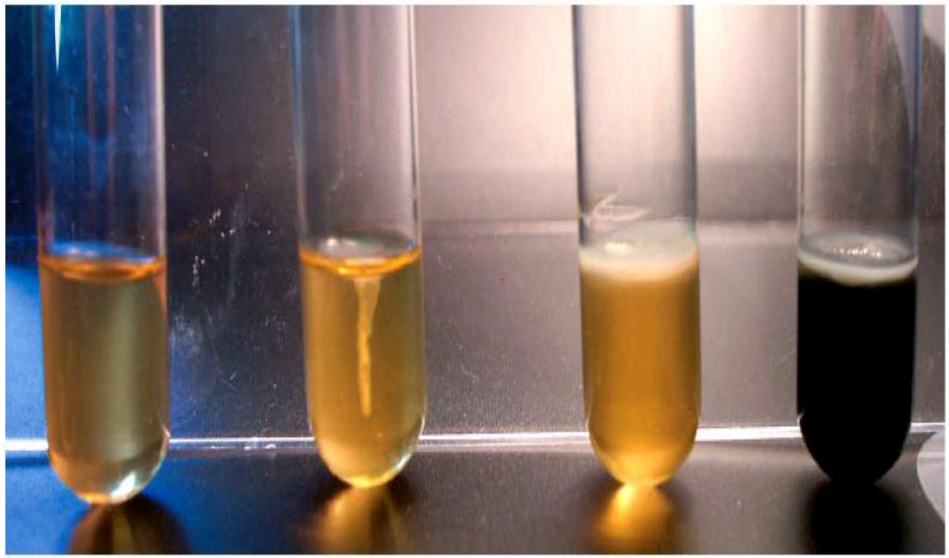
Liquid (broth) used for expanding an organism and for biochemical testing.

The growth appears as turbidity of clear fluid or in the form of sediment or surface pellicle.

Semi-solid media contain <1% of agar It is commonly used to test for motility and to ship Microorganisms from one place to another sometimes termed ‘slants’.



(a)



(b)

Solid media:

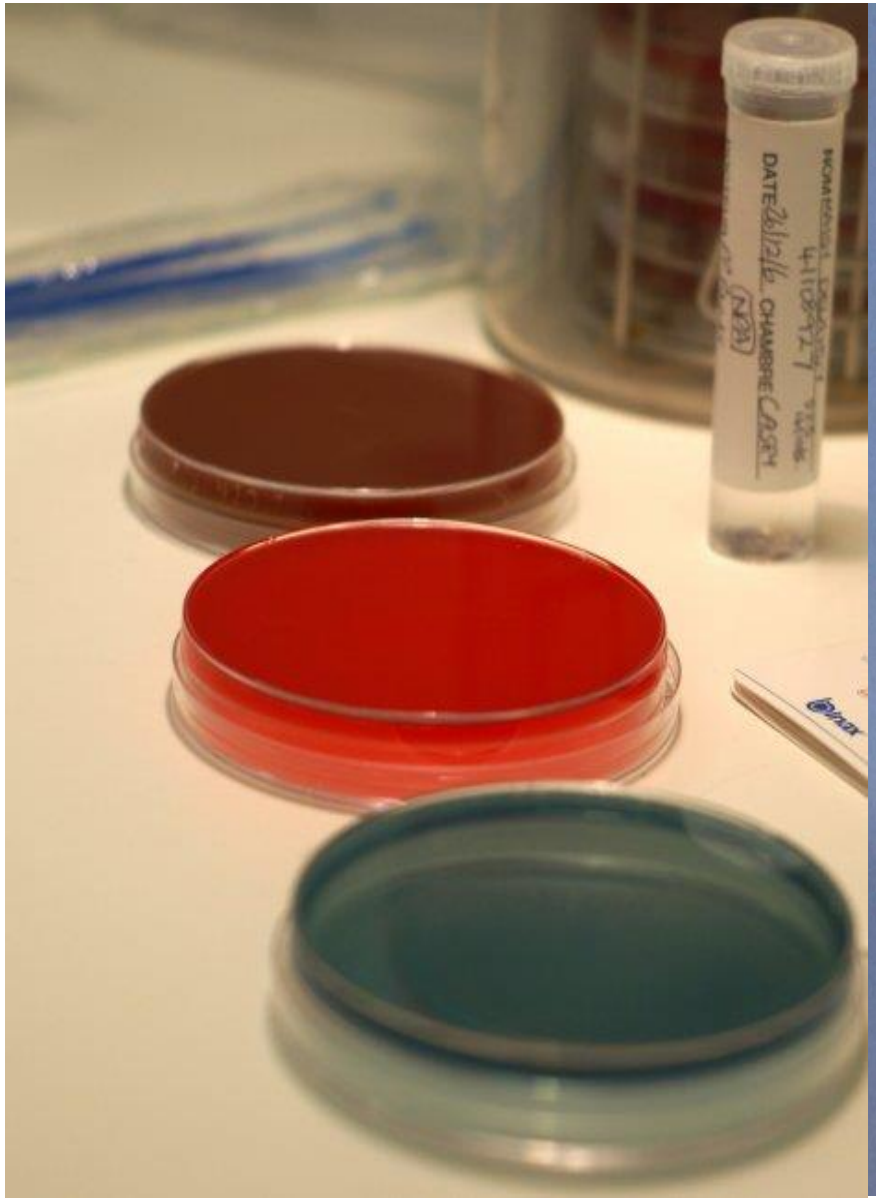
By the addition of agar as a solidifying agent in Petri plates, stabs and slants.

Frau Angelina Hesse, the American wife of one of Pasteur's colleagues suggested adding agar to liquid media to solidify it.

This enabled Koch to grow bacteria in pure cultures.

Advantages of agar:

- **Complex polysaccharide.**
- **Generally not metabolized by microbes.**
- **Liquefies at 100°C.**
- **Solidifies ~40°C.**




Nutrient Agar
Cat #: 11005-008
Lot #: 0533606
Unit: 100mm x 25ml
Store at: 2 to 8 C
Exp. 04/02/06
www.ipmscientific.com
For educational or research use only

Solid media plates used for pure colony isolation and slants for short term storage of organisms.

Significance of "pure colonies":

- MOST NATURAL SOURCES OF microbes consist of a heterogeneous mixture of many kinds of bacteria.
- Any study, either research or clinical, requires (homogeneous) pure strains as a start.

Classification of culture media according to chemical composition:

- Synthetic media (Defined)
- Nonsynthetic or complex media (Undefined).

❖Synthetic media contain pure organic and inorganic compounds that are chemically defined (i.e. known molecular formula).

❖Some media are minimal, some require many more ingredients.

❖For synthetic media – you must know the EXACT growth requirements of a microorganism.

❖**Complex or undefined media** contain ingredients that are not chemically defined or pure (i.e. animal extracts).

❖**Not exact chemical formula**

❖ **Most are extracts from animals: blood, serum, tissue extracts, Yeast extract, soybean extract, digests of casein (milk protein), beef, soybeans, yeast cells ……other highly nutritious but chemically undefined.**

Classification of culture media according to functional types:

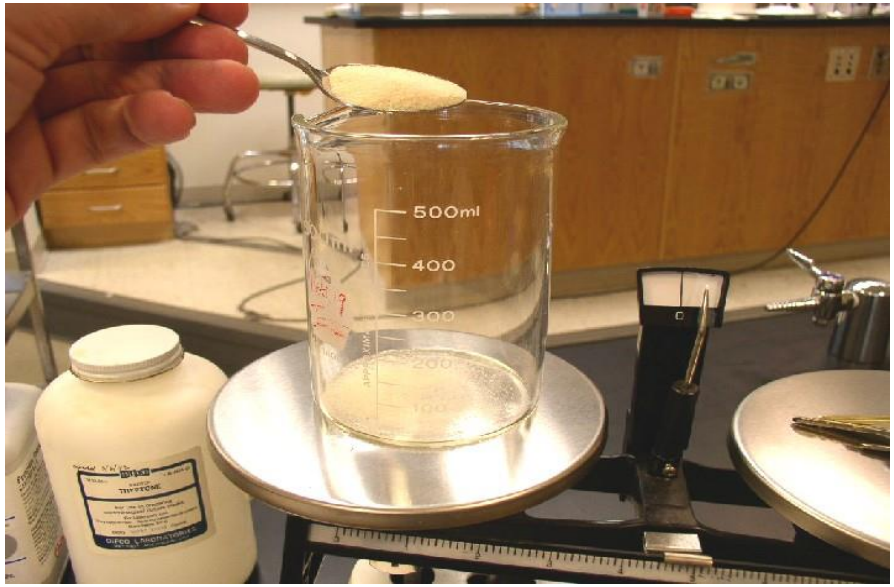
- **Simple (basal) media: contain the essential growth requirements.**
- **Enriched media contain complex organic substances that certain species MUST have to grow – these organisms are often termed ‘fastidious’.**
- **Selective media contain agents that inhibit growth of certain microbes.**
- **Differential media contain growth agents that promote different phenotype of different organisms on same media.**

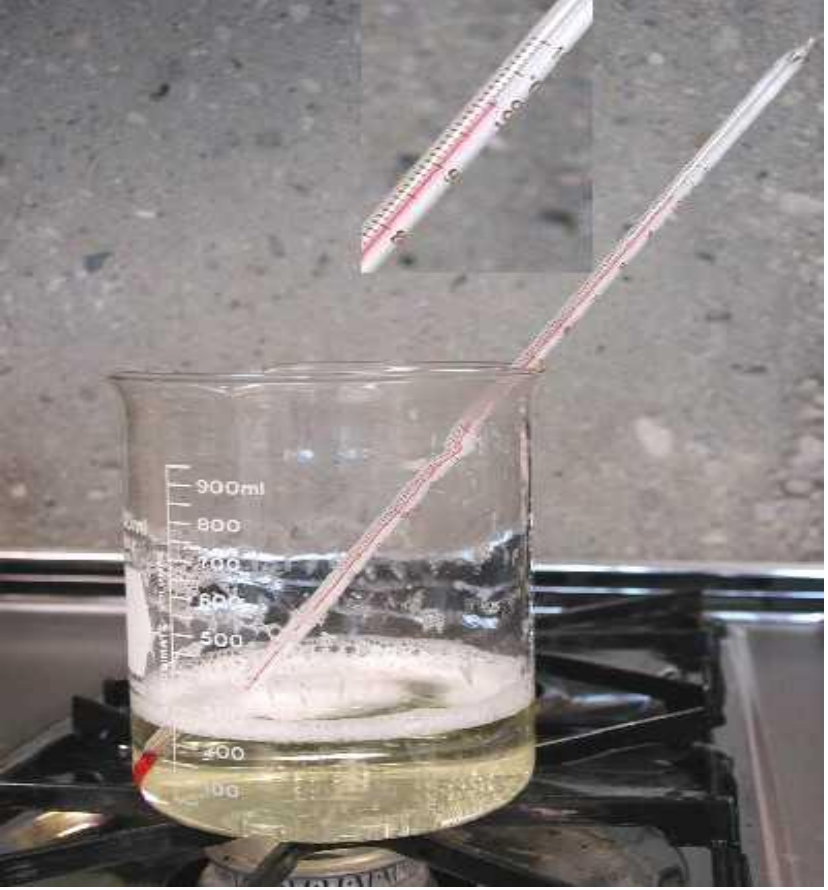
Preparation of culture media

Equipments and supplies required for preparation of culture media

Equipment	Supplies
1000 mL beaker or vessel into which to weigh dry media	
Balance	dehydrated media
spirit thermometer, -10°C to 110°C	600 mL dH2O
heat source, hot pads	16-20 sterile petri dishes
1000 mL bottle with cap	
funnel	
autoclave	

WEIGHING OUT and DISSOLVING THE DRY MEDIA




900ml
800
500


Sterilmatic
Caution
Scalding Steam
Upon Opening!
PRESSURE
TEMPERATURE
FAST
(INSTRUMENTS)
SLOW
(LIQUIDS)
EXHAUST
SELECTOR
1. Fill with 4 to 6 quarts of water.
2. Load sterilizer & close door.
3. Select exhaust.

POURING PLATES

When plates have solidified, invert, place in 37°C incubator for 48 hours to check for sterility and to dry out excess moisture. Store in labelled plastic bag at 4C°. Pre-warm before using.

Sterlization of culture media

- **The complete sterilization of a suitable growth medium so that no living organisms exist at the start of the experiment.**
- **Sealing of the flask was not proper to prevent drying of the media. It was necessary, therefore, to include some kind of filter to prevent the entry of microbes but not of air. So, cotton wool plug, was adopted universally by microbiologists.**

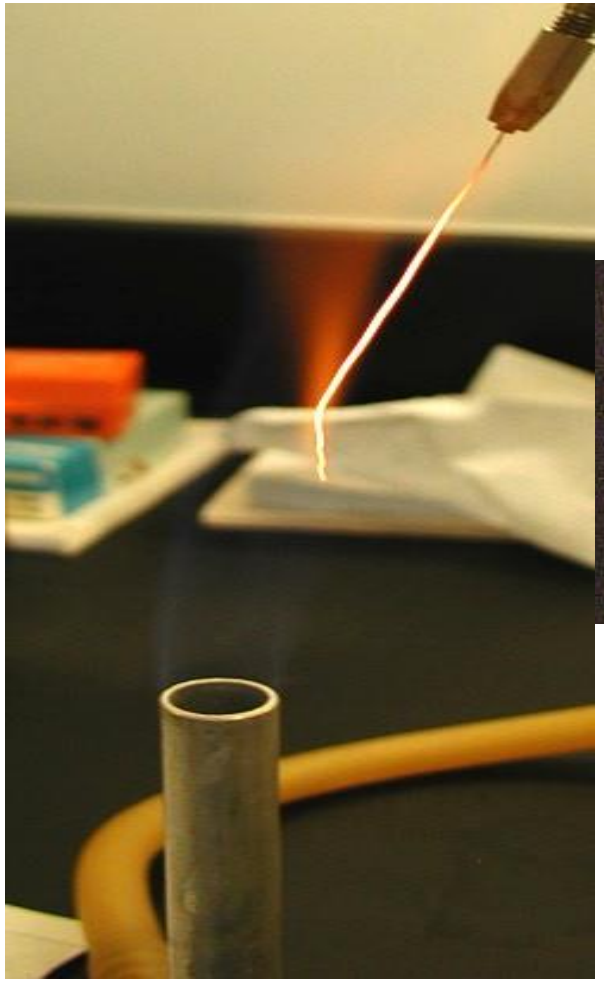
The autoclave: For any culture media not destroyed by heat.

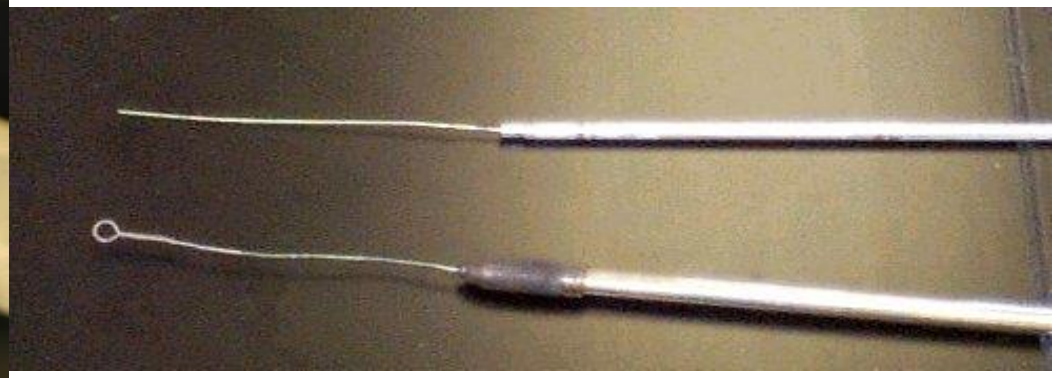
Steaming at 100°C (Koch's sterilizer):

For sterilization of TCBS, Selenite F broth and DCA medium. Tyndallization or intermittent steaming 20 - 45 minutes for 3 successive days is the method used for sterilization of sugar media which decompose at higher temperature.

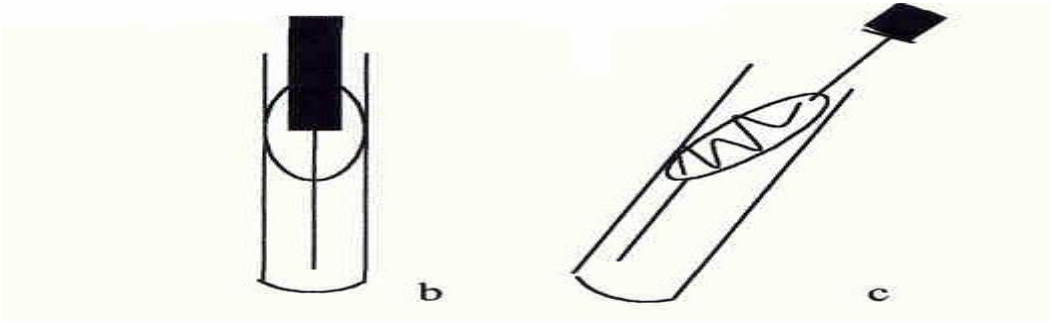
Inspissations: exposure of the medium to humid heat at 75°C for two hours on 3 successive days. This method used for the sterilization of Loffler's serum, Dorset egg and Lowenstien Jensen medium.

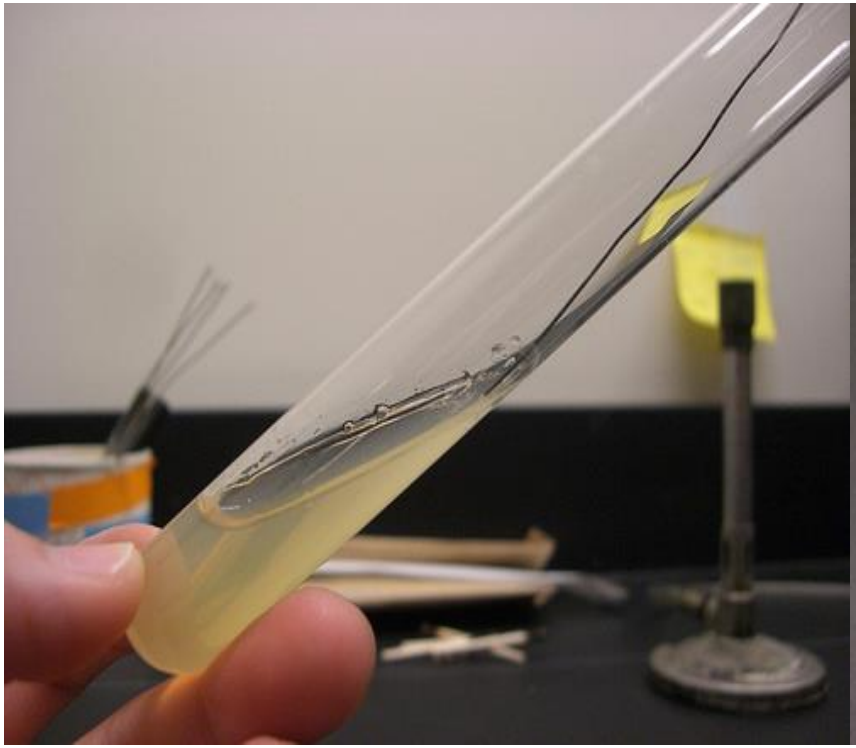
Inoculation of culture media

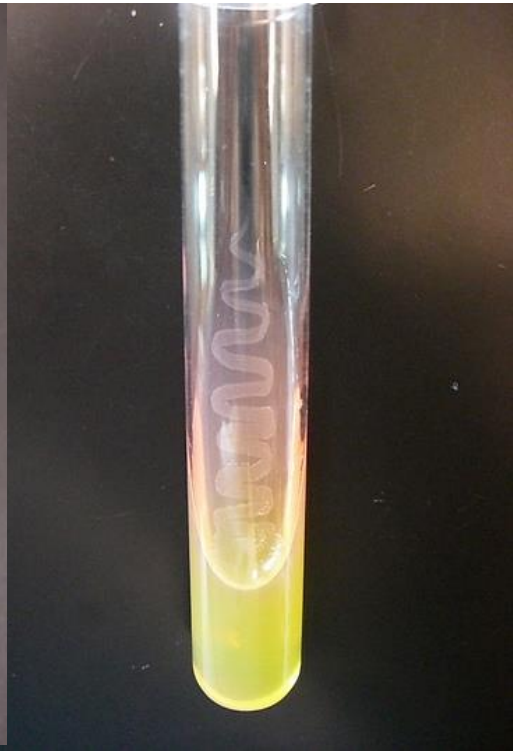




Inoculation of Agar slants



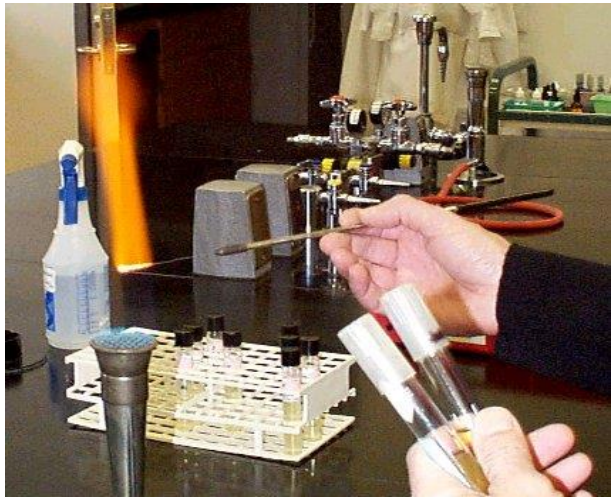




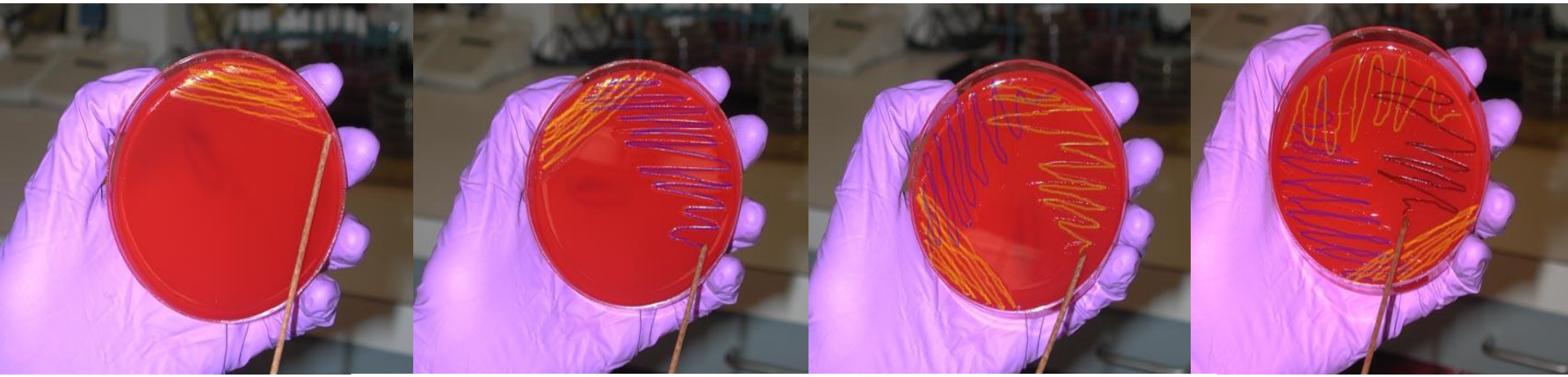
Inoculation of deep Agar Tube

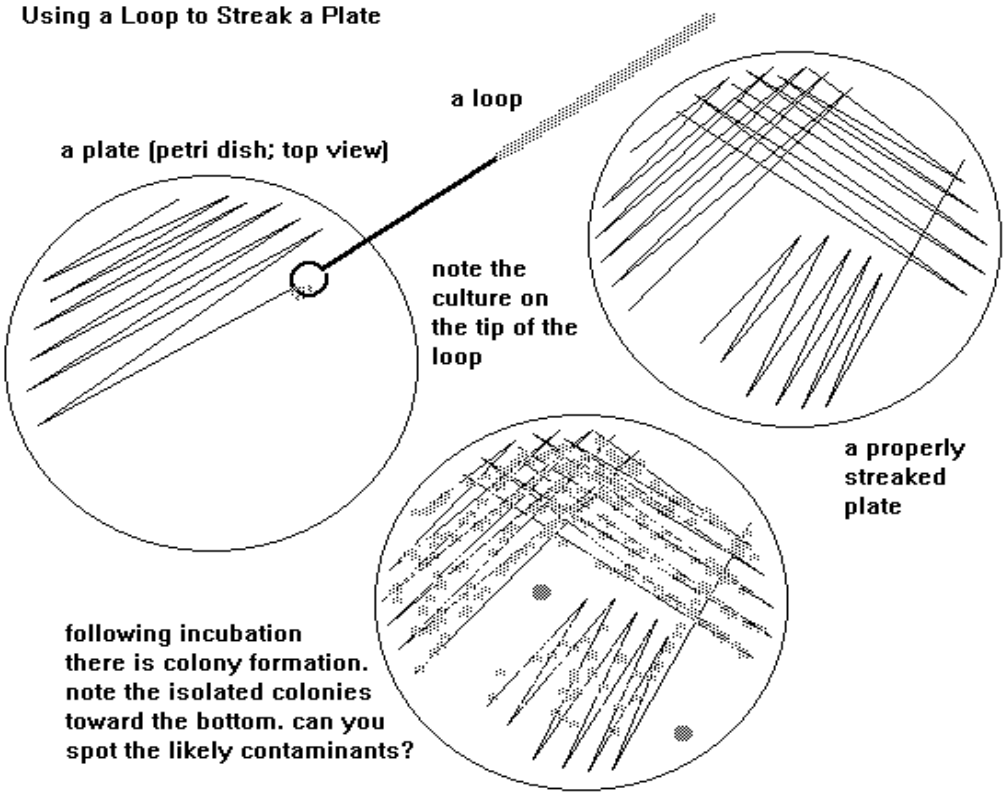
Push needle directly into the agar to the bottom of the tube, then remove it.

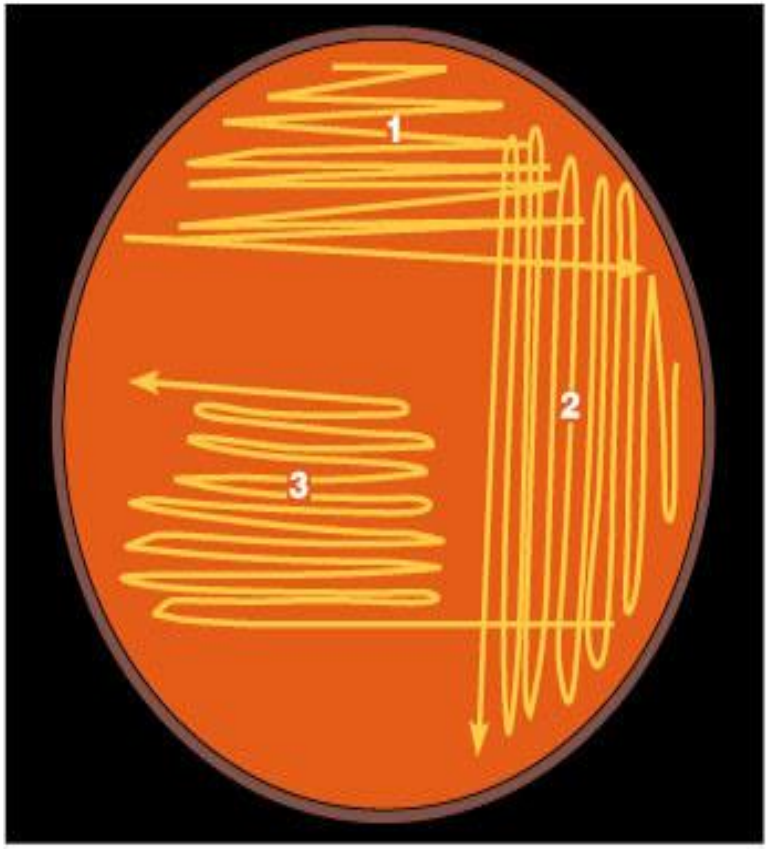
Inoculation of fluid media



Inoculation of soild media







(a) The direction of streaking is indicated by arrows. Streak series 1 is made from the original bacterial culture. The inoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns.

(b) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and white, have been obtained.

Preserving cultures

***Preserving cultures* is important for:**

- **scientific reasons**
- **identification**
- **vaccine production**
- **industrial use**

Methods of preserving cultures

- **Refrigeration**
- **Lyophilization**
- **Freezing**

Refrigeration (4°C)

Effective short term preservation:

Refrigeration can be effective for short periods. Broth cultures, stabs, slants and streak may be *refrigerated*.

Lyophilization

Freeze drying:

Lyophilization is the freeze-drying of cultures. Cultures are first frozen and then dried under high vacuum.

To revive cultures they are rehydrated by broth.

Lyophilization can be an effective long term method of storage.

Freezing

Freezing with protection:

Broth cultures are mixed with various ingredients (e.g., glycerol, DMSO) to limit damage upon freezing and then frozen to temperatures ranging from -50°C to -95°C.

To revive culture they are thawed, and resuspended into broth.

***Freezing* can be an effective long term method of storage.**

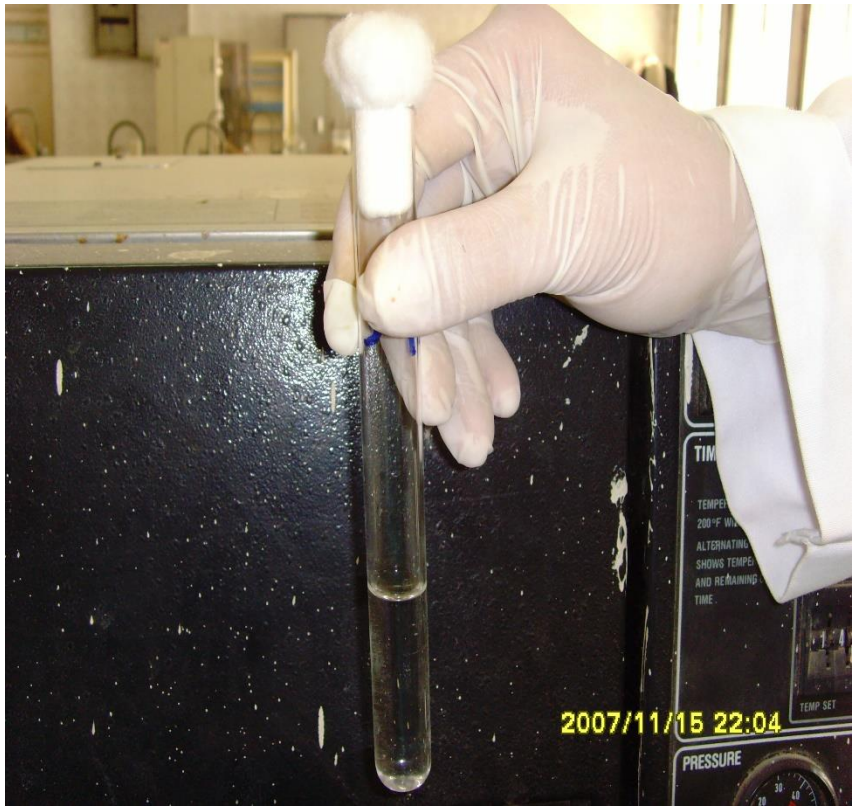
Simple media

It contains the essential growth requirements.
It is used for:

1) **Growing organisms that do not have special growth requirements.**
2) **Base for other media.**

Peptone water

-**Type: Simple fluid media.**
-Composition : peptone + Na Cl +distilled H2O.
Sterilization: autoclave
-Uses: preparation of sugar media and indole Test.
NB: Alkaline peptone water is peptone water in which pH is adjusted at 8-9 and is used a selective and enrichment media for isolation of vibrio cholera from stool.



Nutrient broth

Type: simple fluid media .

Composition: peptone water + meat extract .

Sterilization: autoclave.

Uses:

1) Expanding an organism.

2)Growing organisms that do not have special growth requirements.

3) Base for other media.

Nutrient agar

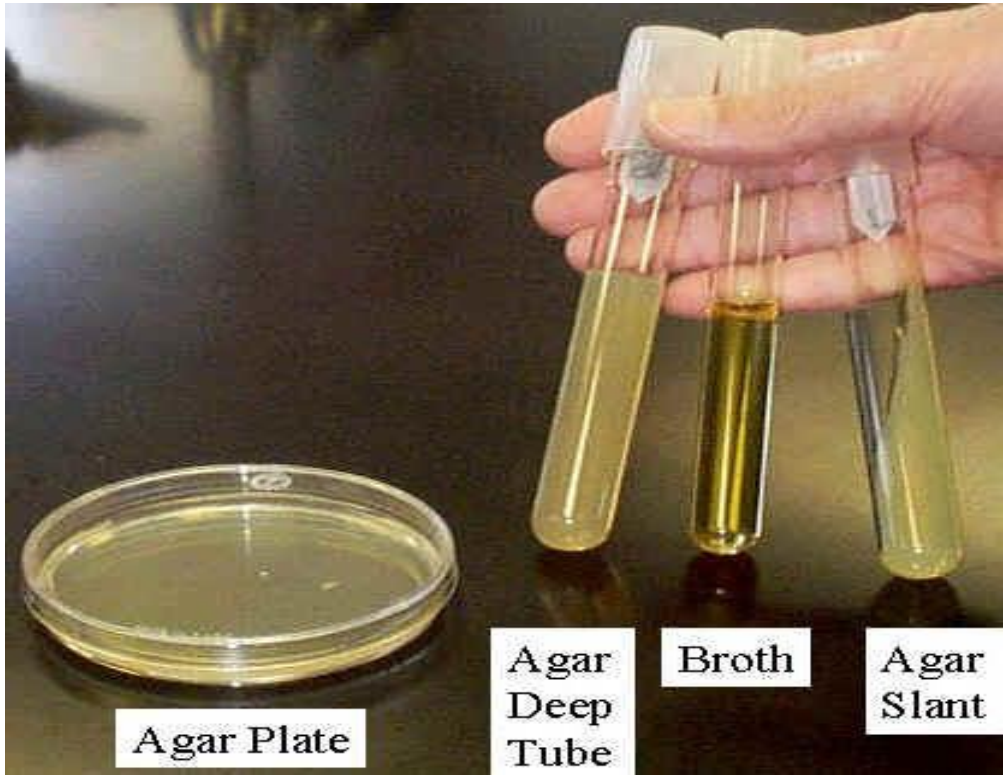
Type: simple solid media (Plate or Slope).

Composition: nutrient broth + 2 % agar .

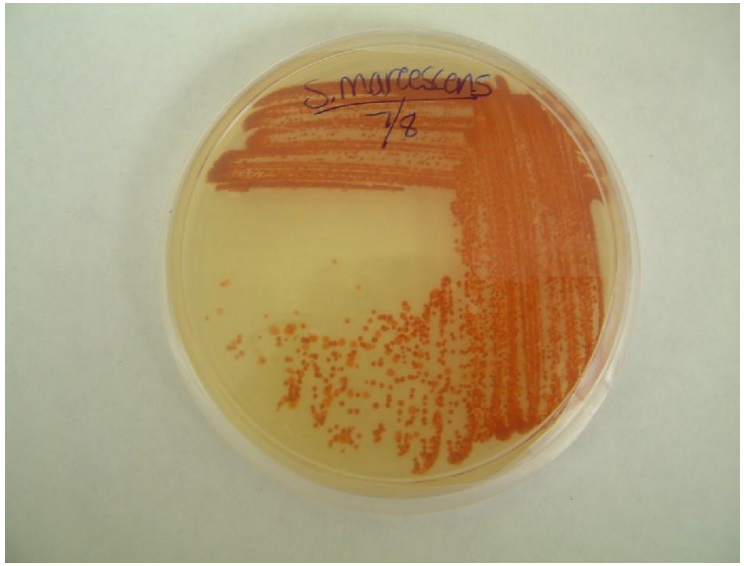
Sterilization: autoclave.

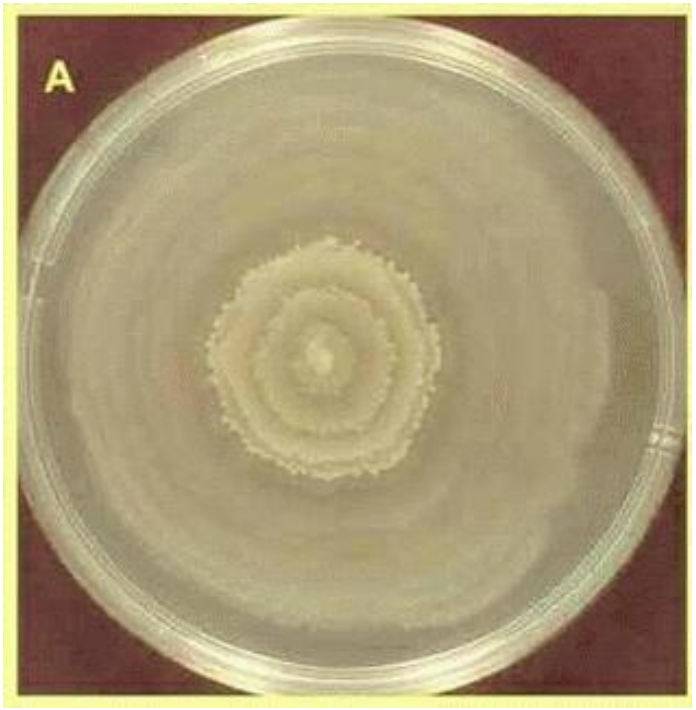
Uses:

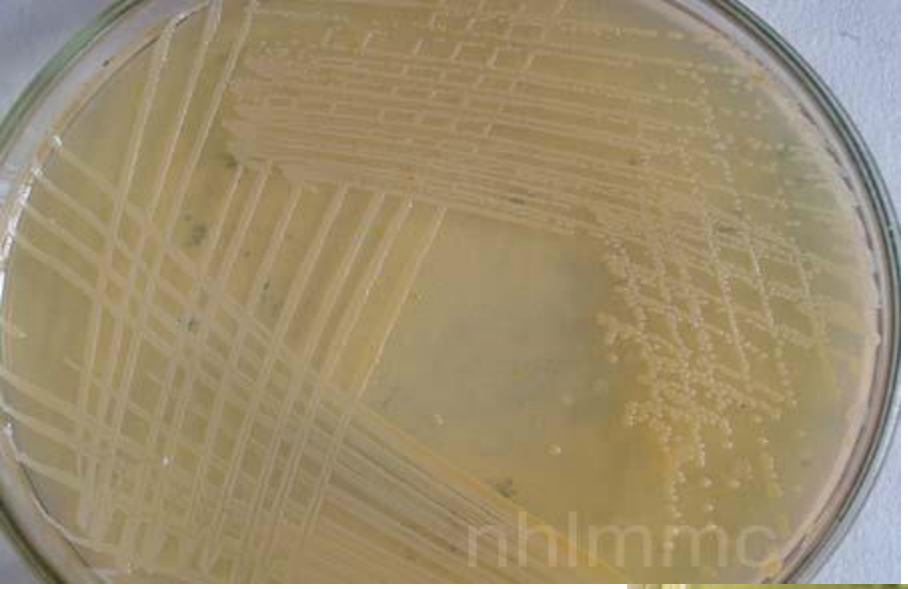
1)Growing organisms that do not have special growth requirements.
2) Base for other media.

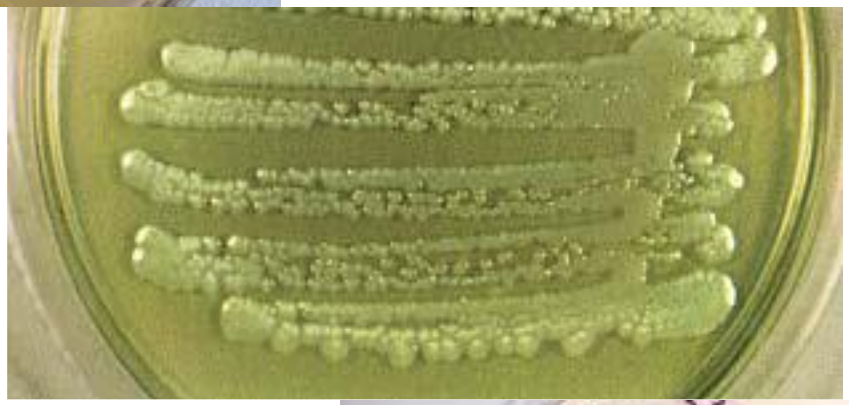

Agar Plate
Agar Deep Tube
Broth
Agar Slant

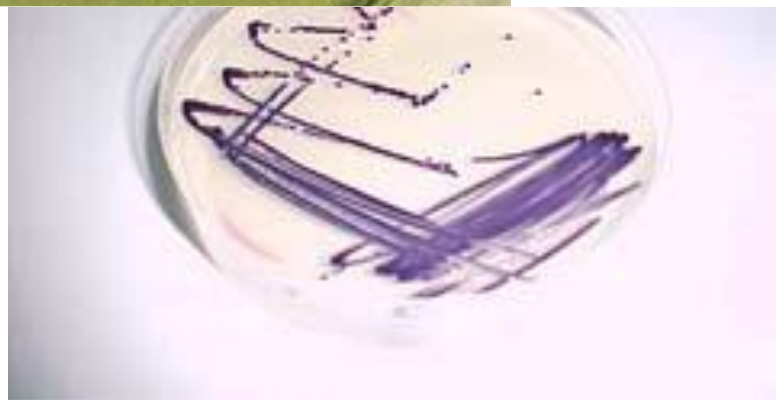
Some organisms have characteristic appearance on it











Enriched media

- **Enriched media contain in addition to the essential growth requirements, highly nutritive substances such as blood, serum or egg.**
- **- It is used for cultivation of the fastidious organisms such as Neisseria and Haemophilus.**

Blood agar

Type: enriched .

Composition: nutrient agar + 10% sheep, horse, ox or human blood.

Sterilization: nutrient agar by autoclave + sterile blood is added to it when its temperature reaches 55°C then it is poured in plates.

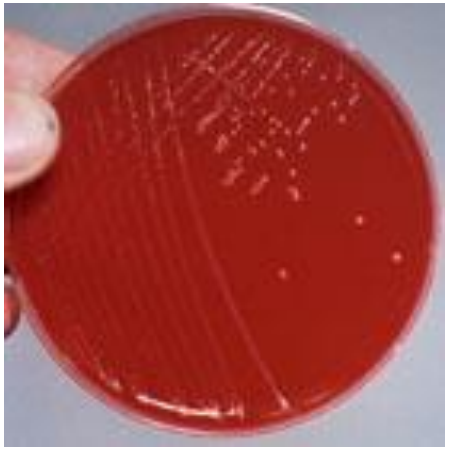
Uses:

1) Cultivation of fastidious organisms as Pneumococci, Neisseria and Haemophilus.

2) Test haemolytic activity of some organisms :

- **Beta haemolytic organisms colonies surrounded by clear zone of complete haemolysis as Streptococcus pyogenes, staph. Aureus & clostridium tetani (they produce haemolysin toxin).**

- **Alpha haemolytic organisms colonies surrounded by greenish zone of incomplete haemolysis as Streptococcus pneumoniae & viridans Streptococci (they don't produce haemolysin toxin).**




β

Chocolate agar

Type: enriched .

Composition: as blood agar

Sterilization: as blood agar but after addition of blood we increase the temperature of water bath to 80 c for 10 min (during heating ,the red cells are ruptured and nutrients as hematin is released).

-Uses: cultivation of Neisseria and Haemophilus.

Loffler`s serum slope

Type: enriched .
Composition: 3 parts of sterile ox, sheep or horse serum + One part glucose broth (nutrient broth + glucose).
Sterilization: inspissation
Uses: cultivation of C. diphtheria

Dorset egg medium (Slope , screw capped)

Type: enriched.

Composition: nutrient broth + whole fresh beaten egg.

Sterilization: inspissation.

Uses: cultivation of T.B from non-contaminated specimen as C.S.F

Differential media

- These media contains a substance that is changed visibly as a result of the metabolic activities of particular organisms.
- Make it easy to distinguish colonies between different kinds of microbes.

MacConkey's agar

Type: differential .

Composition: peptone water + agar + bile salts (inhibit the growth of non-intestinal bacteria) + lactose and neutral red (indicator system). Neutral red is a pH indicator that become pink in acidic media.

Sterilization: autoclave.

Uses: 1- cultivation of enteric bacteria.

2- Differentiation between enteric bacteria:

Lactose fermenters give pink colonies as E.coli. Non lactose fermenters give pale yellow colonies as Salmonella .

Escherichia coli

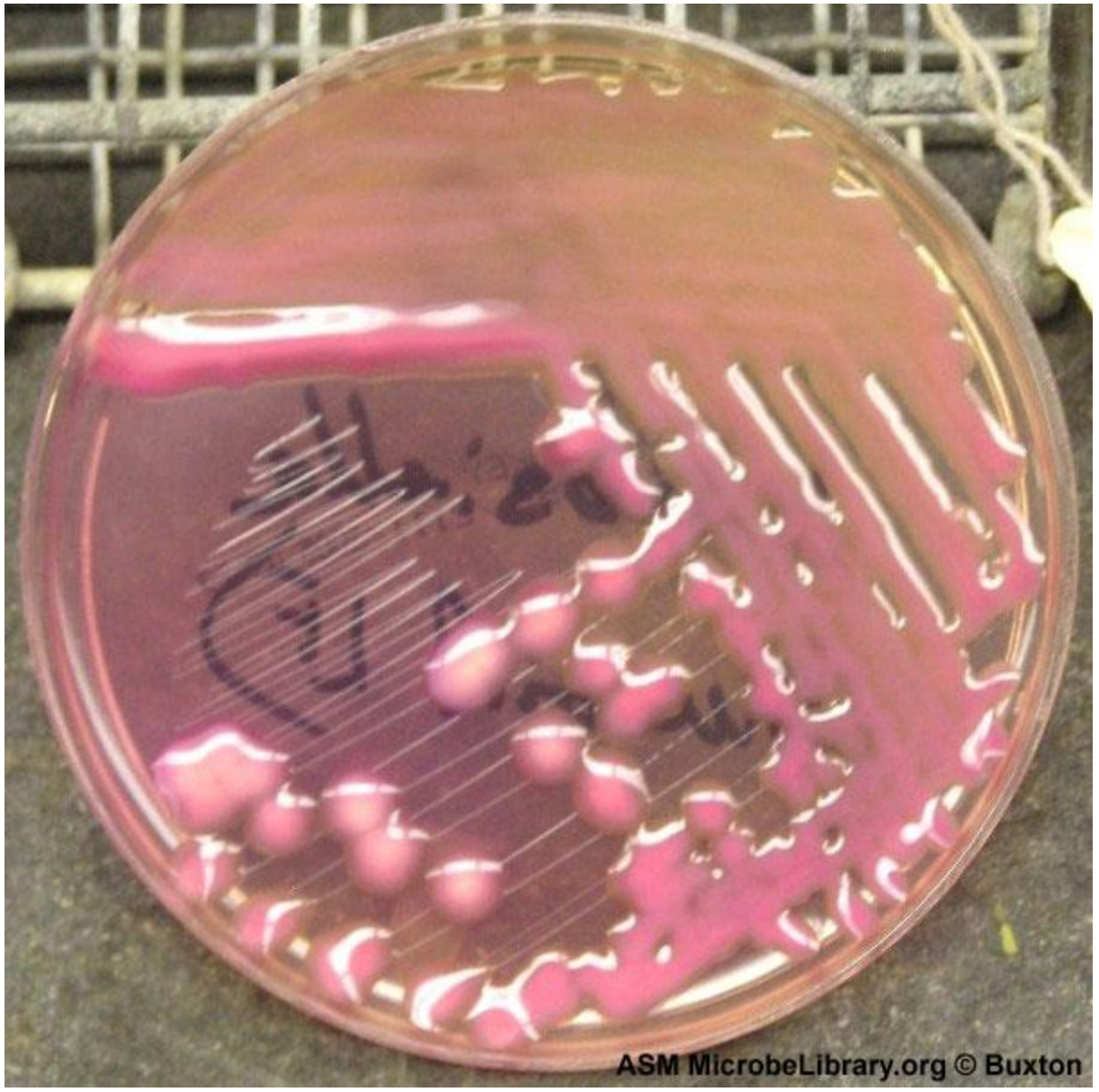
Enterobacter aerogenes

Proteus vulgaris

Salmonella typhimurium

Staphylococcus aureus

MacConkey's Agar


ASM MicrobeLibrary.org © Buxton

Triple sugar iron agar (slope)

Type: differential .

Composition: Nutrient broth, beef extract and yeast extract (nutrients) + soft agar (0.5%) to crack on gas production + 3 sugars: 0.1% glucose, 1% lactose, 1% sucrose and phenol red (indicator system)+Na thiosulphate +Ferrous sulfate (for H_2S detection). Phenol red is a pH indicator which is red in alkaline media and become yellow in acidic media.

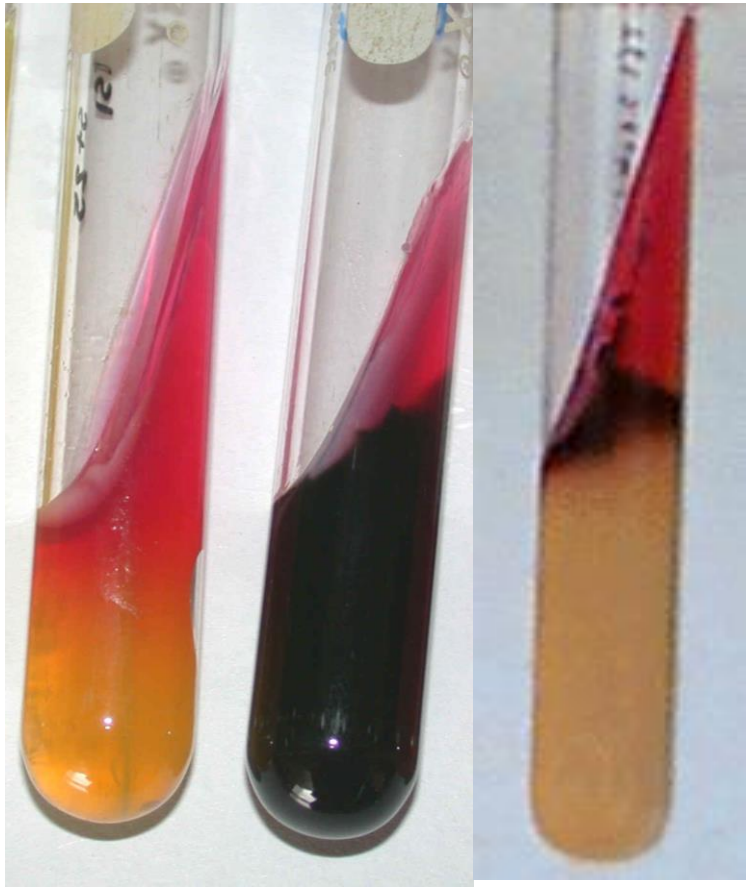
Sterilization: autoclave

Uses:

differentiation of enteric bacteria by testing their fermentative activity:

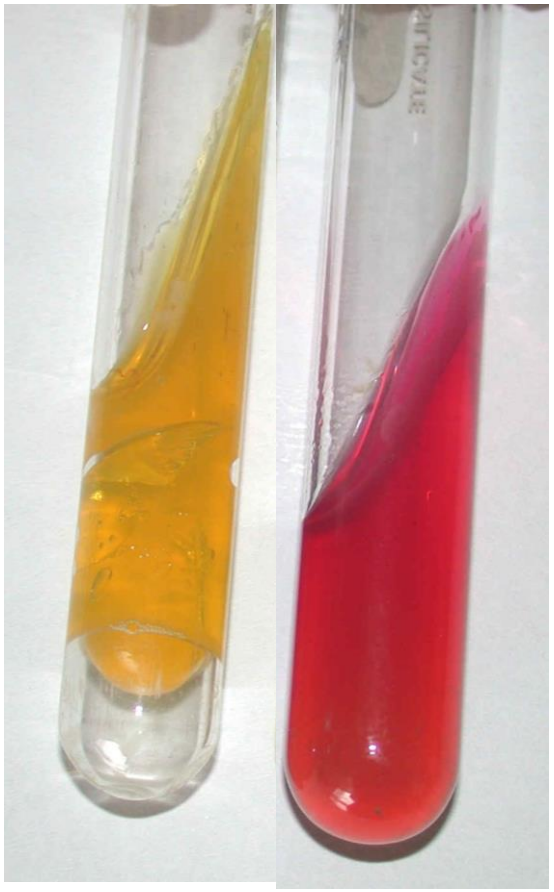
1-Bacteria that ferment glucose only, will release a small amount of acid and give a red slant and a yellow butt e.g. Shigella .

2-Bacteria that ferment glucose only with H2S production will give a red slant and a black butt e.g Salmonella & proteus (If no gas…S.typhi but if with gas….. S.paratyphi B or proteus)



3-Bacteria that ferment glucose and lactose and/or sucrose will release a large amount of acid and gas and give a yellow slant (acid) and a yellow butt (acid) e.g. E. Coli and Klebsiella. Gas appear at the bottom.

4-Bacteria with no carbohydrate fermenting activity will give a red slant (alkaline) and a red butt (alkaline) e.g. Pseudomonas aeruginosa



Mannitol Salt Agar

Type: selective & differential .

Composition: 7.5 % NaCl ,phenol red as pH indicator changes due to acid into yellow.

Sterilization: autoclave.

Uses: 1) Limits growth of many bacteria

2) Differentiation between *Staph aureus and other staphylococci.*

Eosin Methylene Blue

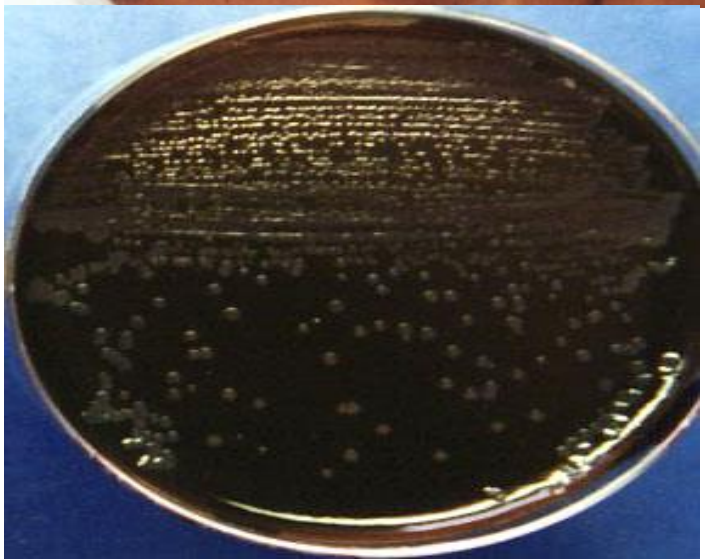
Type: selective & differential

Composition: contains lactose and sucrose, dyes (Eosin Y and Methylene blue) inhibit Gram (+) bacteria.

Uses:

1) Selective for Gram (-) bacteria

2) Distinguish between lactose fermenting (colonies with dark centers and clear borders as green metallic sheen) and non-lactose fermenting organisms (completely colorless colonies).




For
You

Selective media

They contain inhibitory substances that inhibit organisms not desired to grow while the organism under study will grow (selective agents)

Lowenstien Jensen media (L.J) (slope,screw capped)

Type: selective.

Composition: whole beaten eggs + mineral salt + malachite green.(selective agent)

Sterilization: inspissation

Uses: Cultivation of tubercle bacilli from contaminated specimen as sputum, urine and stool

Blood tellurite (Mcleod's)

Type: selective .

Composition: blood agar + potassium tellurite 0.03% as A selective agent.

Sterilization: as blood agar.

Uses:

1) Isolation of C. diphtheria from throat swabs as it inhibits most of the normal flora of the URT.

2) Differentiation of the types of C. diphtheria due to the varying black to gray color they produce.

Thayer-Martin medium (Ch. + VCN)

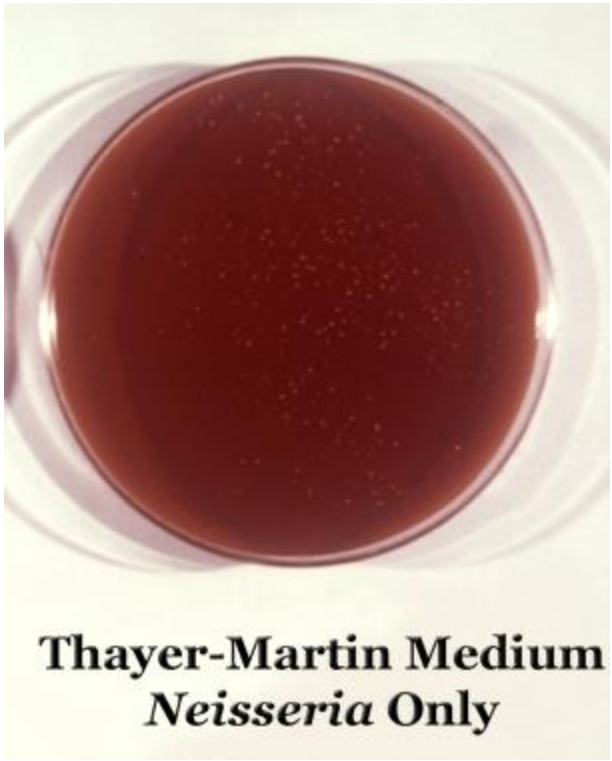
Type : Selective.

Composition: Chocolate agar + three antibiotics; vancomycin, colistin and nystatin as selective agents.

Sterilization: as chocolate agar.

Uses: cultivation of Neisseria gonorrhoeae.

N.B) Thayer-Martin media is not suitable for the isolation of Neisseria meningitides because vancomycin inhibite10% of their strains.



Deoxycholate citrate agar (DCA):

Type : Selective and differential .

Composition: nutrient agar + neutral red and lactose (indicator system) + bile salts (selective agent).

(Neutral red is a pH indicator that become pink in acidic media.

Sterilization: steaming at 100°C.

Uses*:* **1) selective for isolation of shigella, the salmonella food poisoning group and S. paratyphi from stool. They are non-lactose fermenting giving rise to pale yellow colonies.**

2) Differentiate between H2S producers that give pale yellow colonies with black center from non-H2S producers) that give pale yellow colonies without black center.

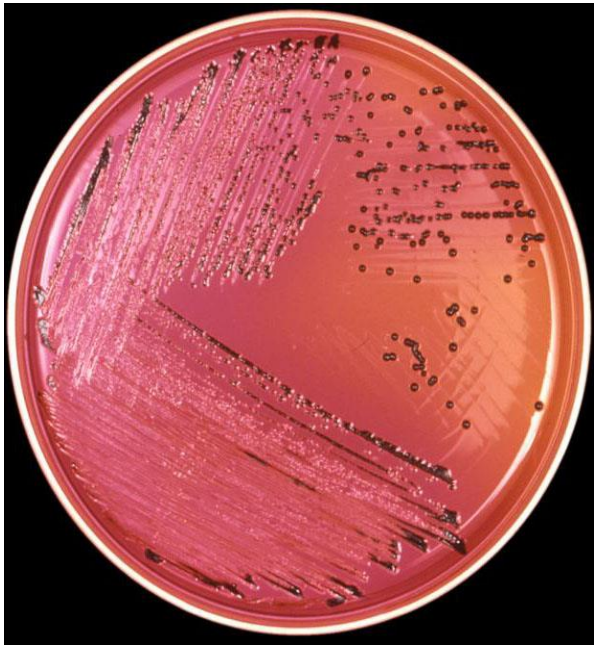
3) If any of lactose fermenting organisms grow on this media, they could be differentiated by the colonies color whish is pink.

T.C.B.S (Thiosulphate-Citrate- Bile salt-Sucrose)

Type : Selective and differential .
Composition: nutrient agar + Na thiosulphate, Na citrate and Bile salt (selective agents) + sucrose and bromothymol blue (indicator system).
Sterilization: steaming at 100°C.
Uses: 1) Selective for isolation of Vibrio species
2) Differentiation between different Vibrio species.

Xylose lysine desoxycholate agar (XLD) agar

Type : Selective and differential .
Composition: Phenol red, Sodium desoxycholate ,Sodium thiosulphate, Ferric ammonium sulphate, Xylose ,Lysine ,Lactose And Sucrose
Uses: Isolation and differentiation of salmonella and shigella from other gram – enteric cocci.



Sugar media

Composition*:* **peptone water + 1% tested sugar as glucose, sucrose, lactose or maltose + andrad's indictor (pH indicator which is colourless in alkaline pH and become red in acidic pH) + Small inverted Durham's tube is immersed in the media for gas collection.**

Sterilization: Tyndalization.

Uses: test the fermentative activity of bacteria:

*Bacteria that ferment any type of sugar with production of acid only will turn the tube red.

*Bacteria that ferment any type of sugar with production of acid and gas will turn the tube red with gas bubbles detected in Durham's tube.

*Bacteria that ferment no sugar the tubes remain colourless.

Simon’s citrate agar

Type: selective medium

Composition: Citrate, ammonium ions, bromothymol blue as a pH indicator which is green at acidic pH turns blue at alkaline pH. Organisms growing on Simmons Citrate Agar are capable of utilizing citrate as the sole carbon source Utilization of the ammonium salt will lead to the formation of ammonia (NH_3) and ammonioum hydroxide (NH_4OH), both of which will alkalinize the medium. The increase in pH then causes colour change in the bromothymol blue indicator, turning it blue.

Enrichment media

- **Special types of selective media grows bacteria that is hard to grow in mixed culture.**
- **Encourage the growth of desired microbe in a mixed culture.**
- **In particular, enrichment media is designed to increase very small numbers of the desired bacteria.**
- **Enrichment culture is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium.**
- **Unlike selective media, enrichment culture is typically used as broth medium.**

Selenite F broth

Type: selective .
Composition: peptone water + Na selenite (selective agent)
Sterilization: steaming at 100ºC.
Uses: *enrichment* media for isolation of salmonella and shigella from stool (inhibit coliform bacilli).

Tetrathionate broth

Type: selective.
Composition: nutrient broth + thiosulphate solution (selective agent).
Sterilization: autoclave.
Uses: as Selenite F broth .

Brain heart infusion broth

Brain heart infusion broth (or BHI broth) is a highly nutritious general-purpose growth medium for **fastidious microorganisms**, such as streptococci, pneumococci and meningococci. It is made by the recuperation of nutrients from boiled cattle hearts and brains. Soluble factors are released into the broth during the boiling procedure. **Sterilization:** autoclave

N.B. It also enriches non fastidious organisms. While N.broth enriches only non fastidious organisms.

Mueller Hinton agar

It is an microbiological growth medium that is commonly used for antibiotic susceptibility testing. It is also used to isolate and maintain Neisseria and Moraxella species.

It typically contains: beef infusion, casein hydrolysate, starch

Five percent sheep blood may also be added when susceptibility testing is done on Streptococcus species.

Media for cultivation of fungi

Sabouraud dextrose agar

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide the nitrogen and vitamin source required for fungi. The high concentration of Dextrose is included as an energy source. acidic PH allows selectivity of fungi.

Blood culture

Direct plating of the blood specimen on culture media for isolation of bacteria causing bacteraemia or septicaemia is not recommended because:

- Organisms are circulating in a few numbers in the blood stream.
- Blood may contain antibodies or any inhibitory substance that will interfere with bacterial growth.
- The patient may take antibiotics that inhibit bacterial growth.

So for culturing of blood specimens, blood culture bottles containing 50-100 ml of sulphonated broth are needed.

- The big volume of broth has the following advantages:

- **It dilutes out antibodies or natural antibacterial substances in the serum.**
- **It provides good nutrient condition for multiplication of the organism..**
- **If the patient is taking antibiotics; antagonists to that antibiotic can be added to the fluid medium e.g. penicillinase is added if the patient is taking penicillin.**

Na sulphonate is used as an anticoagulant .

There are several blood culture systems are commercially available:

1. **Monophasic blood culture medium: the bottle contains only sulphonated broth (any additional nutrient is added according to the growth requirements of the isolated organism). Subculture is done from this culture every 48hs on suitable solid media and blood culture is considered negative only if no growth appear after 10 days.**
2. **Diphasic blood culture medium (Castenada) that combines a broth medium (liquid phase) with a solid agar medium (solid phase) in the same bottle.**

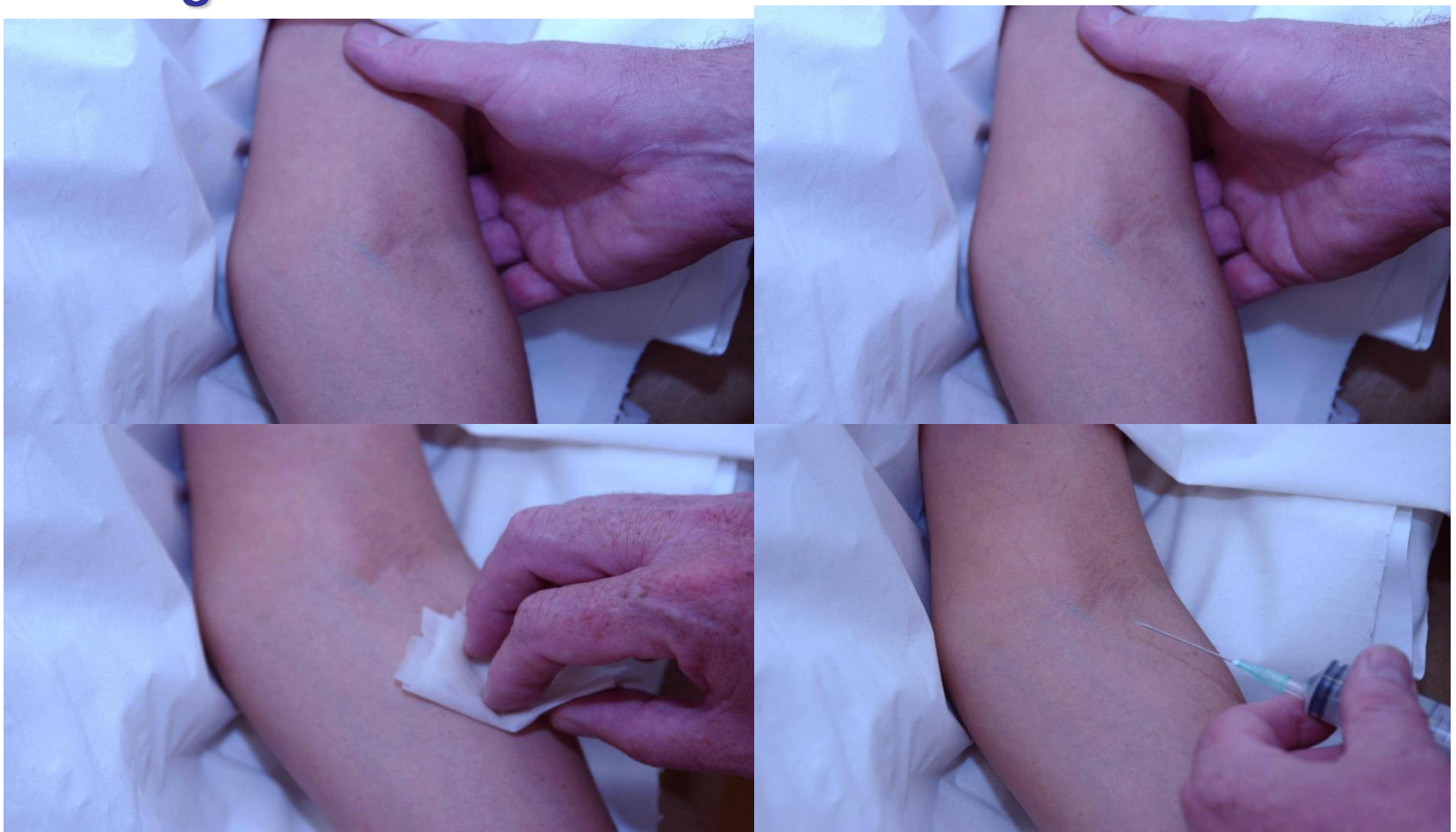
It has the advantage of decreasing the incidence of contamination present in monophasic type from repeated subculture as the subculture is done on the agar in the same bottles.

3Automated blood culture systems that monitor early signs of bacterial growth are also available.

Indications of blood culture :

1. Subacute bacterial endocarditis.
2. The diseases in which bacteraemia is one of its features as enteric fever, brucellosis, meningitis, puerperal sepsis, relapsing fever, epidemic and endemic typhus.

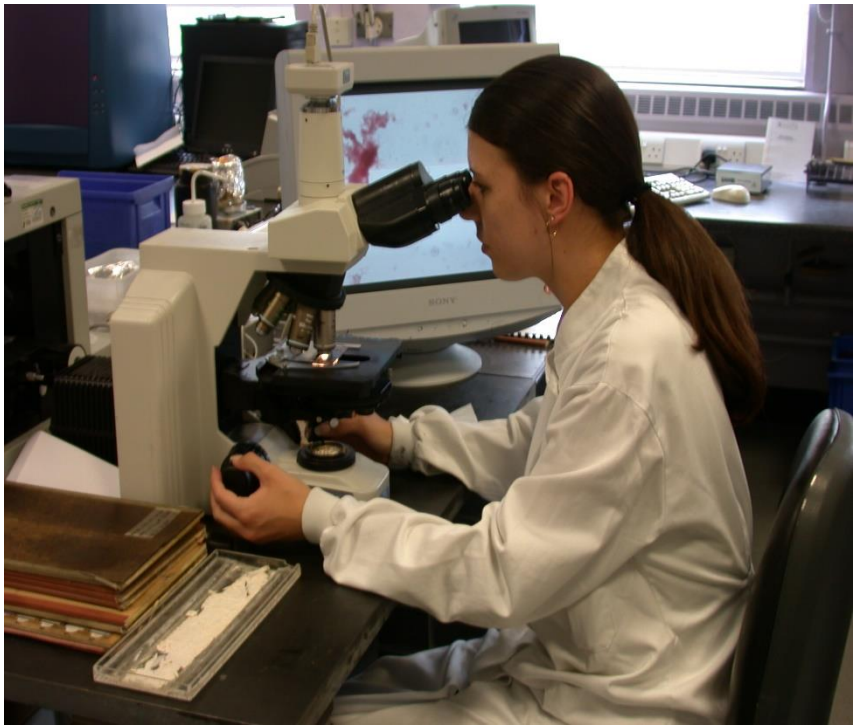
Collecting blood cultures: Inform the patient of the procedure; apply the tourniquet and select the vein for puncture: wash your hands/use alcohol hand rub and then clean the puncture site with an alcohol wipe; enter the vein without palpating the site again.



Before collection ensure that all requirements are at hand (before venepuncture, remove caps, wipe septa with an alcohol wipe, and allow to dry). After collection of blood, fill the anaerobic bottle first, so that any air in the syringe is not inoculated into that bottle.

Dispose of sharps and other items safely. Blood culture bottles should be placed in any ward incubator until collected by the porters for transport to the laboratory. (Ensure that the request form is complete, with clear clinical details, before placing it in the plastic bag with the bottles)

The fluid from the positive bottle is also used to inoculate agar plates on which most bacteria will grow. The inoculum is also spread over a susceptibility agar plate, on which are placed antibiotic discs appropriate for the organism seen on the gram stain. This will give the "direct" sensitivity results.



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THANK YOU