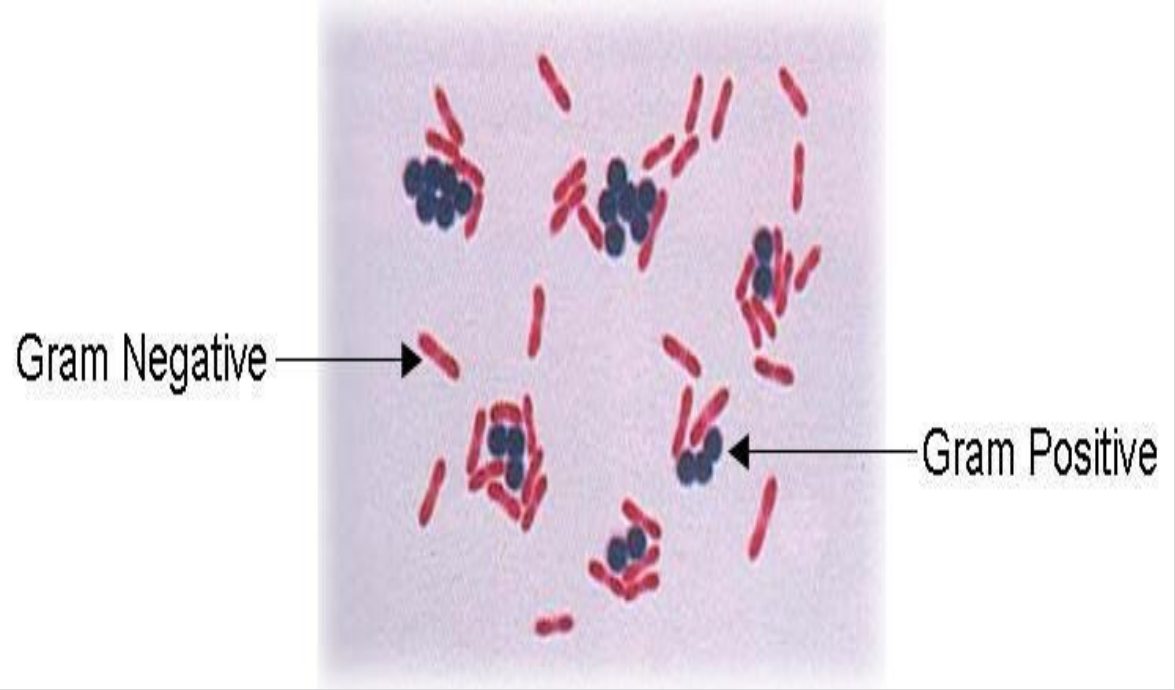
Microbiology lab (1)

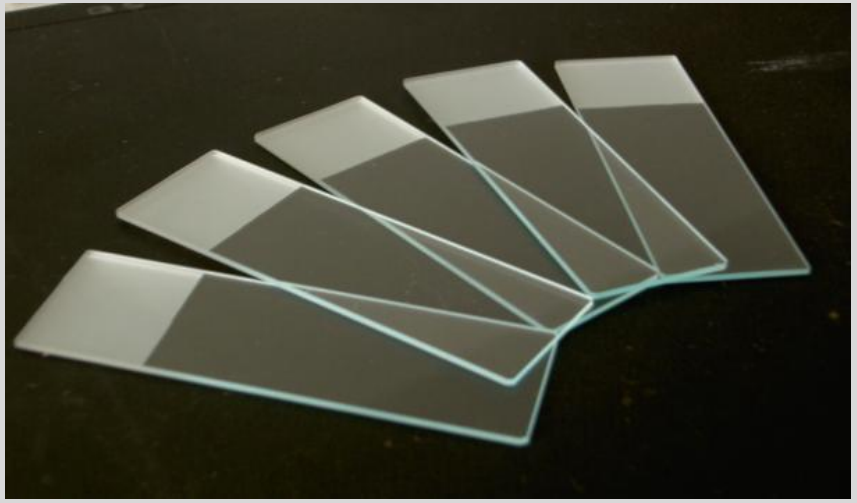
Done by: Abdelhadi Okasha

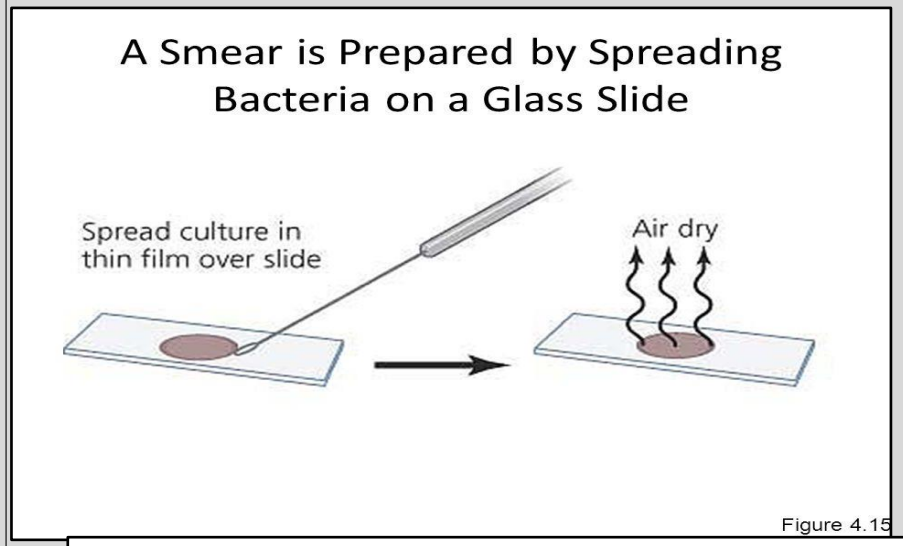
- Some of the common safety rules in labs:
  1) sterilize equipment and material
  2) disinfect working area before and after working using Dettol and alcohol
  3) Don't eat or drink in the lab
  4) Keep the flame open to sterilize air around
  5) Wear a clean lab-coat
  6) Wash your hands with soap and water before and after working
  7) Dispose lab wastes properly

- Gram stain: a differential staining technique that uses a primary stain and a secondary counter stain to differentiate between gram positive and gram negative bacteria
  → Gram positive: violet or blue
  → Gram negative: Pink
  - Note: this test is useful not only to distinguish between gram positive & negative, but also to know the shape & the arrangement of the bacteria

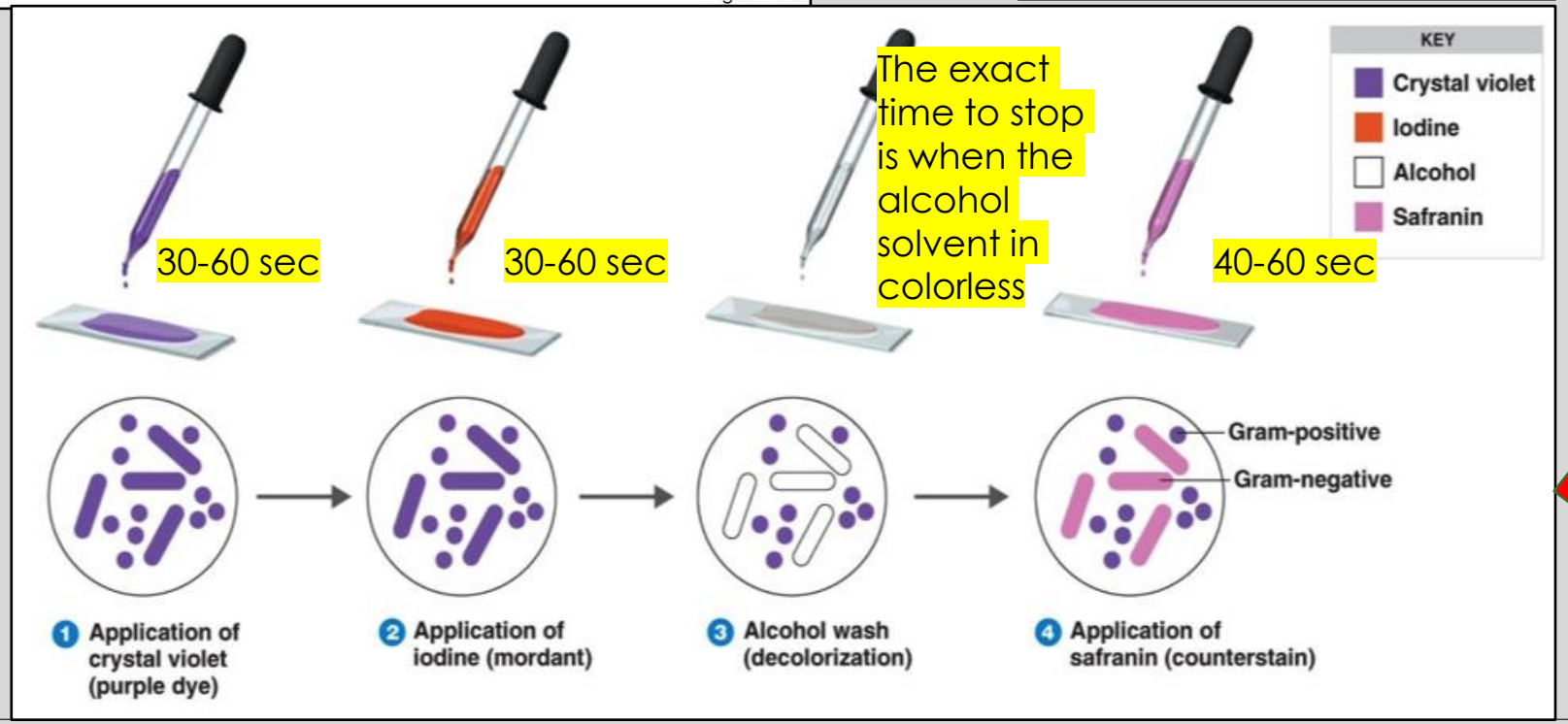


- Gram stain stools are:
  1) crystal violet stain
  2) 70% ethyl alcohol
  3) gram iodine stain
  4) safranin stain
  5) slide
  6) wire loop
  7) flame

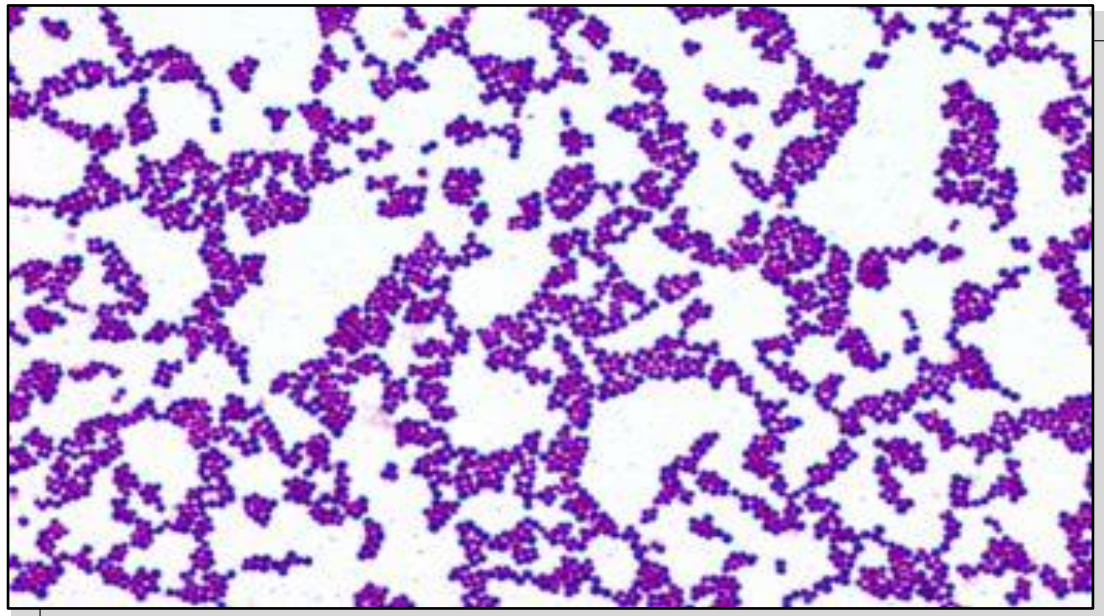




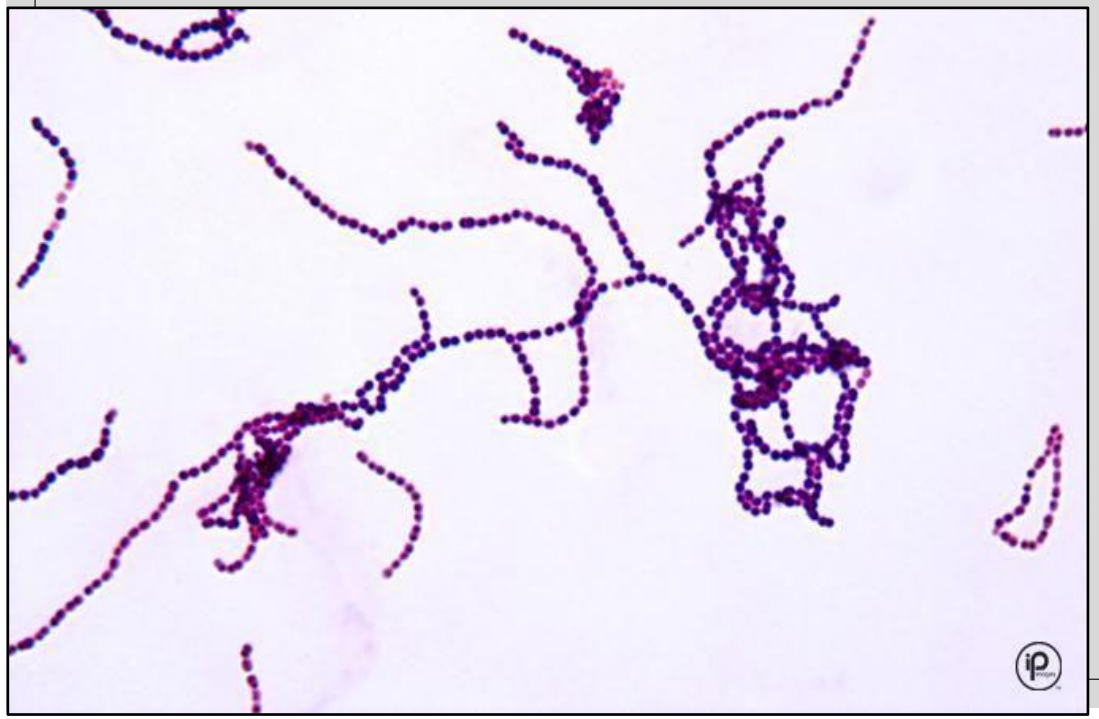
→ We move slide on flame in a circular way
→ Heat help in adhesion of bacteria on the slide



→ After each staining process, we remove the excess stain with a stream of water, and then shake the excess water from the surface
→ Last step we can use a towel or hot air to remove excess water



→ Color: Gram positive
→ Shape: Cocci
→ Arrangement: Clusters



→ Color: Gram positive
→ Shape: Cocci
→ Arrangement: Chains

→ Color: Gram negative
→ Shape: Rods

→ Color: Gram positive
→ Shape: Rods

Microbiology lab (2)

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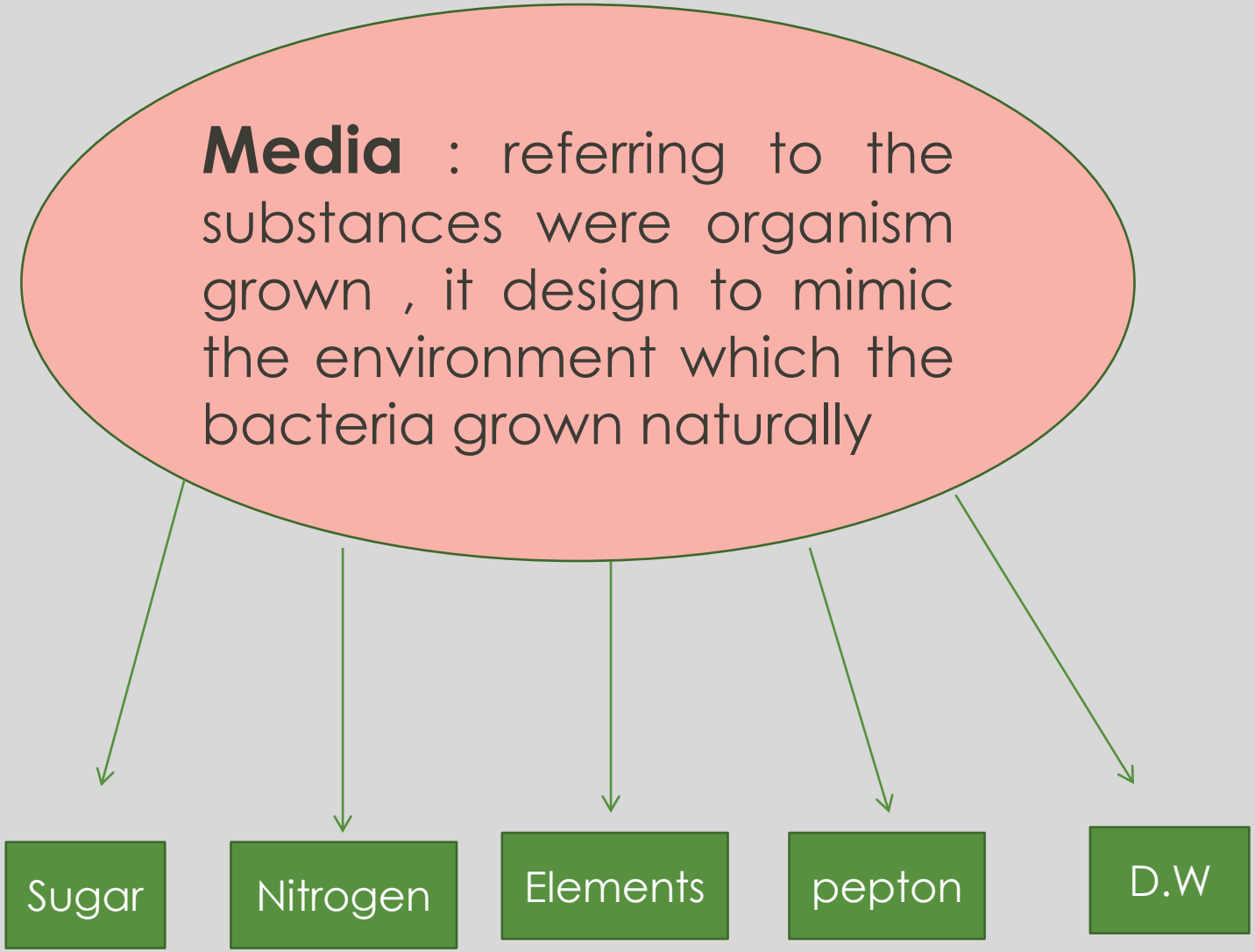
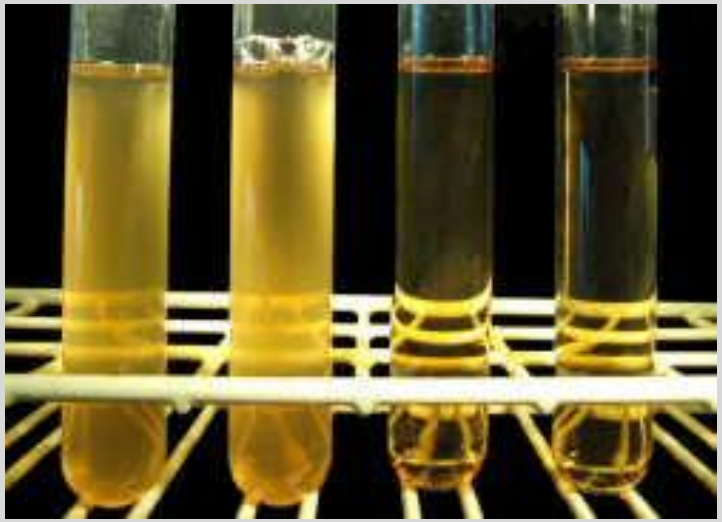
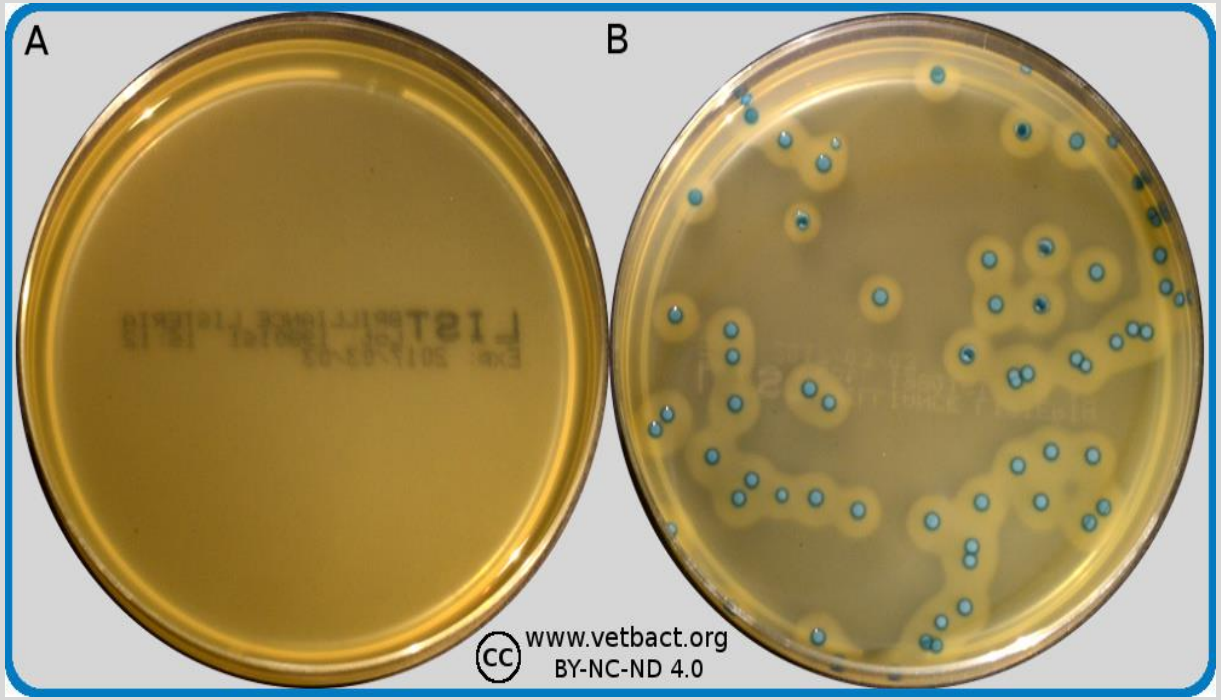

Media : referring to the substances were organism grown , it design to mimic the environment which the bacteria grown naturally
Sugar
Nitrogen
Elements
pepton
D.W

Liquid media (broth): Contain a specific amount of nutrients but doesn't contain gel agents such as gelatin or agar
→ Functions: propagation of a large number of organisms, fermentation studies, and various other tests such as sugar fermentation tests
→ Remember: Growth of bacteria turn the liquid from a clear state into a turbid (مغبش) state



Agar media (Solid media): contains agar at concentration (1.5-2)%, it allows bacteria to grow in a beneficial way because of it's properties (e.g. colonies or streaks)
→ Function: Isolating bacteria + determining the colony characteristic of the isolate




C.L.E.D. Agar
500g XLD
Mueller Hinton II Agar
500g Blood Agar Base


A&D EK-610i
NET
ZERO
10.45 g


OXOID
CM0003
NUTRIENT
AGAR


STIRRING
POWER
HEAT
HEATING
MAGNETIC STIRRER

- **Culture media steps:**

1) Select culture media recipe from data base
2) Calculate ingredient quantities regarding the required culture media volume
3) Weigh main ingredients into the container, then weigh trace ingredients on a high accuracy balance and add to the container
4) Use ionized water up to 80% of the required volume
5) Mix to dissolve the ingredients, gentle heat may be required
6) Top up the culture media to the required volume, then label the container and sterilize it in an autoclave

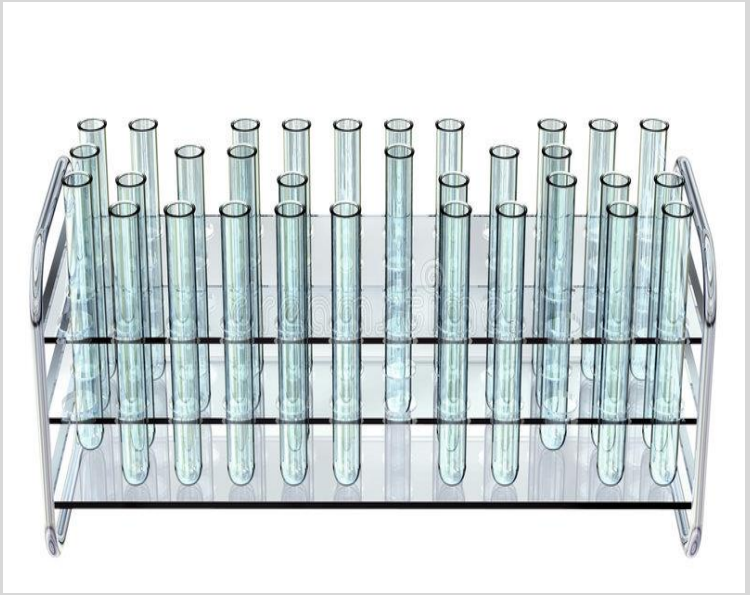
Note: Culture media steps must be carried in a careful way to prevent contamination

- **Autoclave condition**: A device that uses steam under pressure to kill harmful bacteria, fungi, viruses & spores on items that are placed inside a pressure vessels

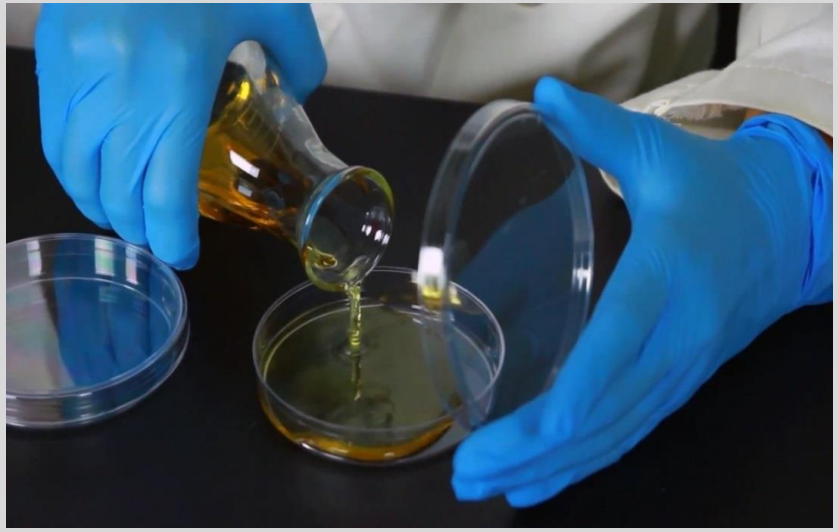
- Temperature:121 C (250 F)
- Time: 30 Min (The doctor mentioned in the lecture that the time is 15-20 min, however, time varies depending on the pathogen contaminating the tool
- Pressure: 15 pound/inch

- Note: Some objects can't be sterilized by autoclave because they contain structures that can be denaturated by temperature & pressure

Liquid media (broth)



Agar media



7) After heating by autoclave is finished, get out the media and check that the color of the autoclave tape changed into black lines.
Remember: Autoclave tape is an adhesive tape used on media when heating in autoclave device to indicate whether a specific temperature (121 C) has been reached
8) Put a sterilized thermometer in the mixture and monitor until it reaches a temperature below 47C

9) For a solid agar, fill the dish about it's quarter and then replace the lead immediately, allow it to cool at room temperature and change into a gelatin state
10) If it's a liquid media, pour it into sterilized tubes
11) The media must be placed in the refrigerator to cool (but not freeze) in an inverted position with a lead in the bottom (to prevent condensation from dropping into the surface of the agar)

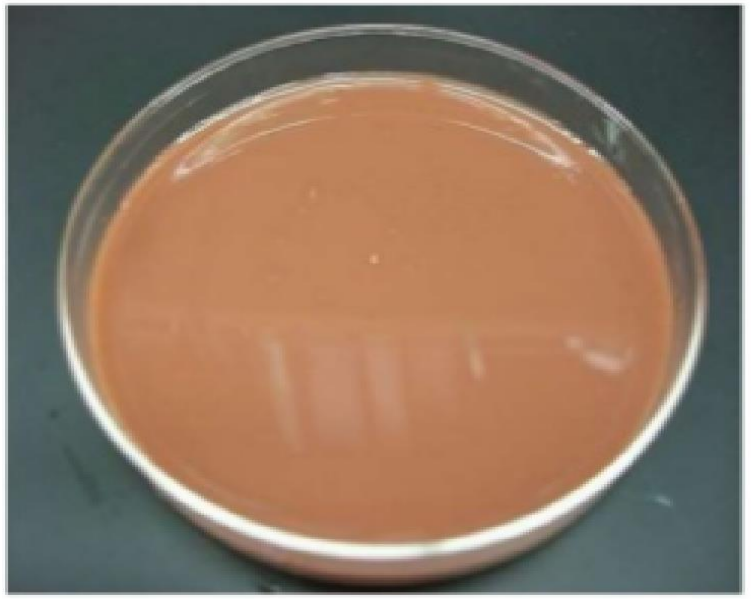
Enriched media

- Contain nutrients important for growth of a variety of organisms
- Used for many fastidious micro-organisms

Blood agar

BAP contains mammalian blood(usually sheep or horse) typically at a concentration of 5-10%,+ soya agar used to isolate fastidious organisms and detect hemolysis.

e.g. of bacteria Streptococcus

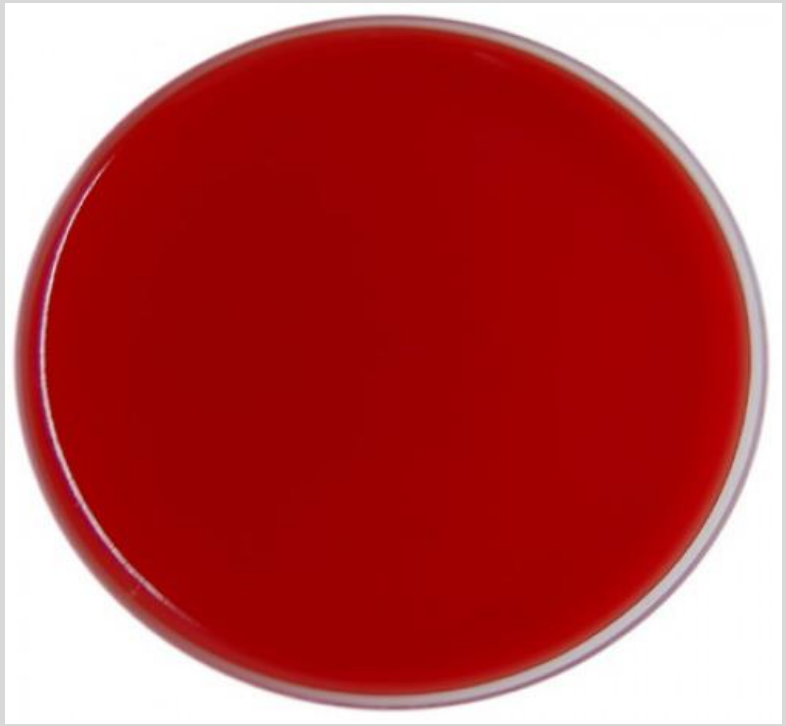


Chocolate agar (Non selective)

contain red blood cells that have been lysed by slowly heating to 80 c .and it used for growing fastidious bacteria, such as *Haemophilus influenzae* + Neisseria species

- Note: Lysing of blood release intracellular co-enzyme: Nicotinamide adenine dinucleotide (NAD), also called 3 factor that is utilized by bacteria

Enrichment media



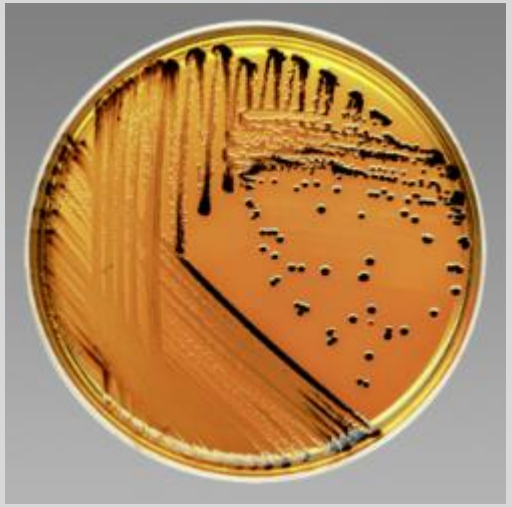
Selective & differential media

- Selective media generally selects for the growth of a desired organism, stopping the growth of or altogether killing non-desired organisms. Differential media takes advantage of biochemical properties of target organism(s), often leading to a visible change when growth of target organisms are present

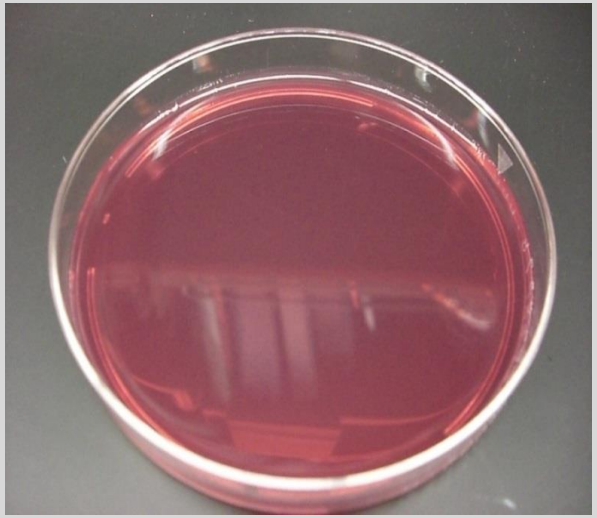
Selective media

- Both SS agar & MacConkey agar are considered as selective & differential media

→ MacConkey is used to isolate & differentiate between enteric microbes by their ability to ferment lactose
- Bile salts and crystal violet inhibit G.ve+ from growth
- Lactose helps in differentiate and also prevent forming of proteus species



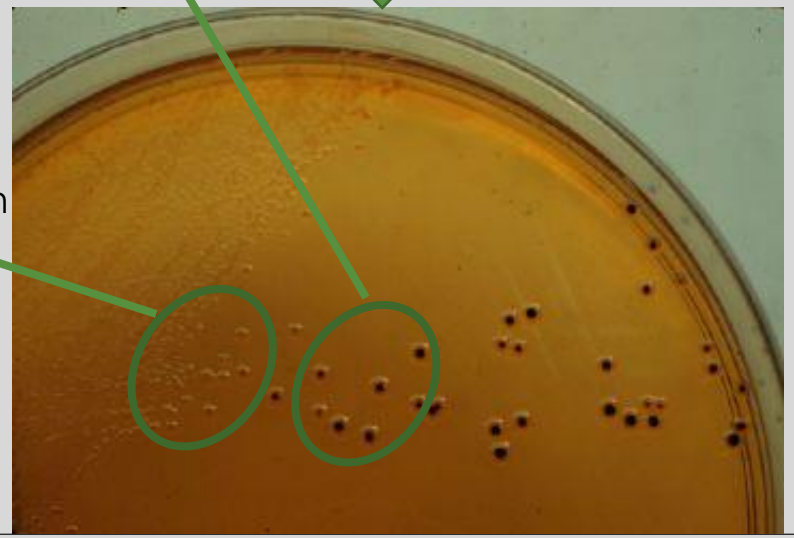
→ SS agar is used for isolation, cultivation and differentiation of gram negative enteric micro-organisms salmonella & shigella species that are isolated from both clinical and non-clinical specimens (e.g. feces, urine, blood)

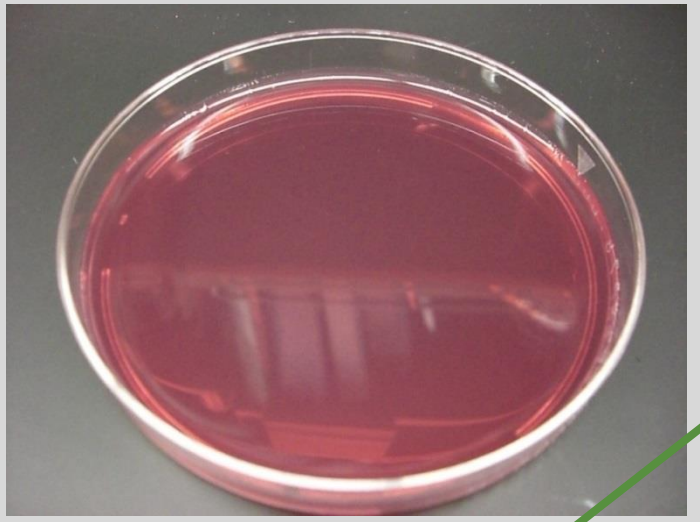


Macconkey agar media

Salmonella (black is because of H2S production

Shigella: No H2S production





Lactose fermenters

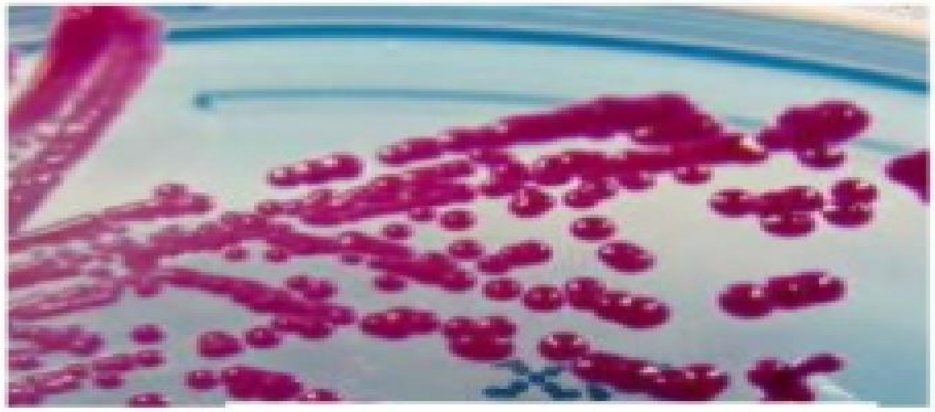
Non-Lactose fermenters

Differential media

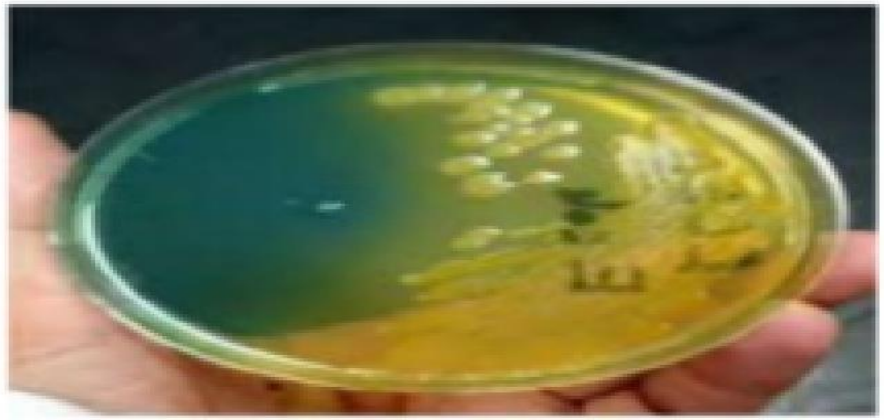
Cysteine Lactose Electrolyte Deficient Agar(CLED)

- For cultivation of pathogen from urine specimen , inhibit swarming of *proteus sp*.

→ Lactose fermenters appear yellow to green, while non-lactose appear white colony

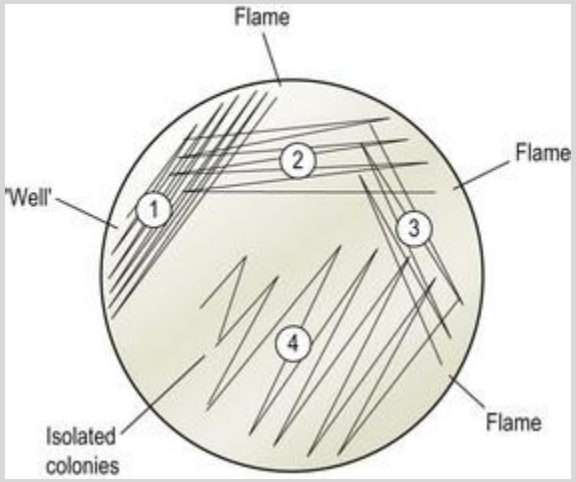


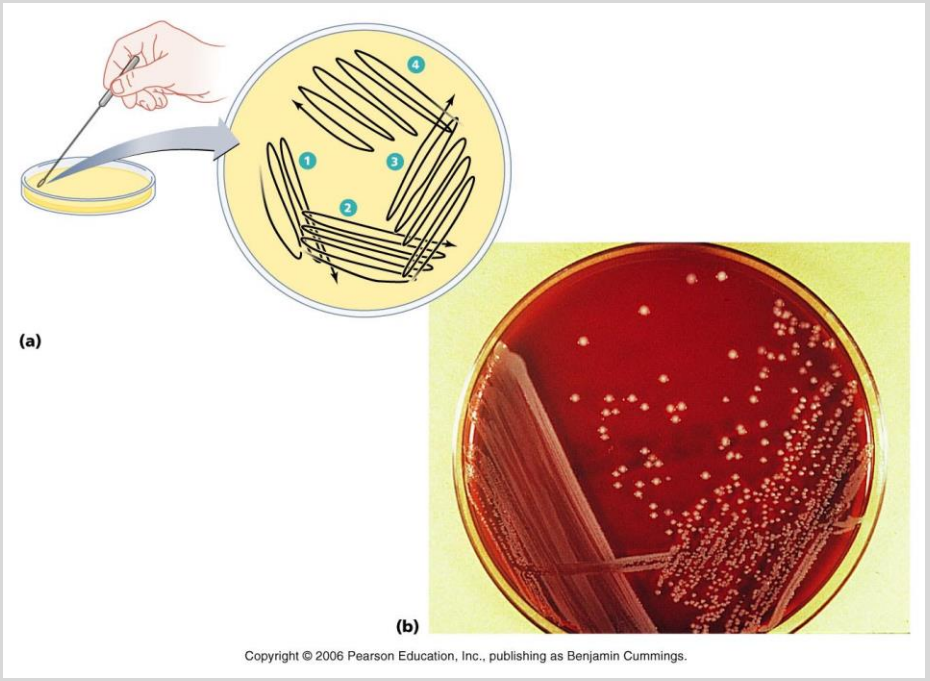
CLED, serratia

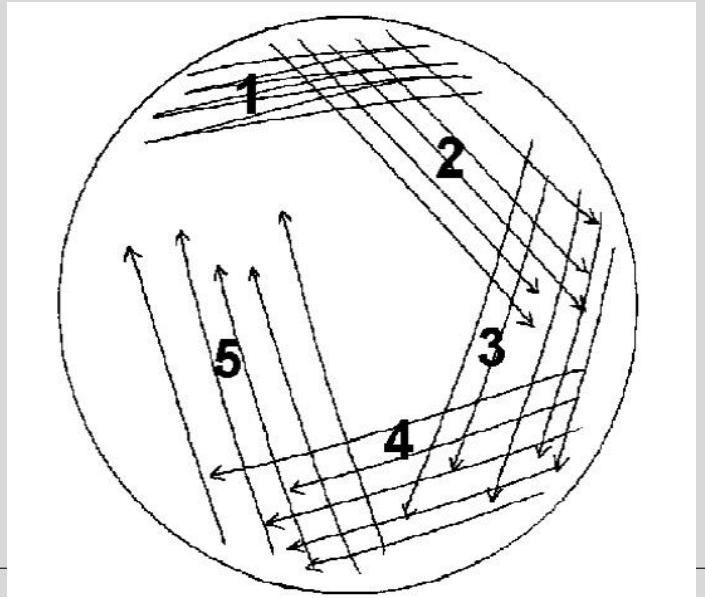


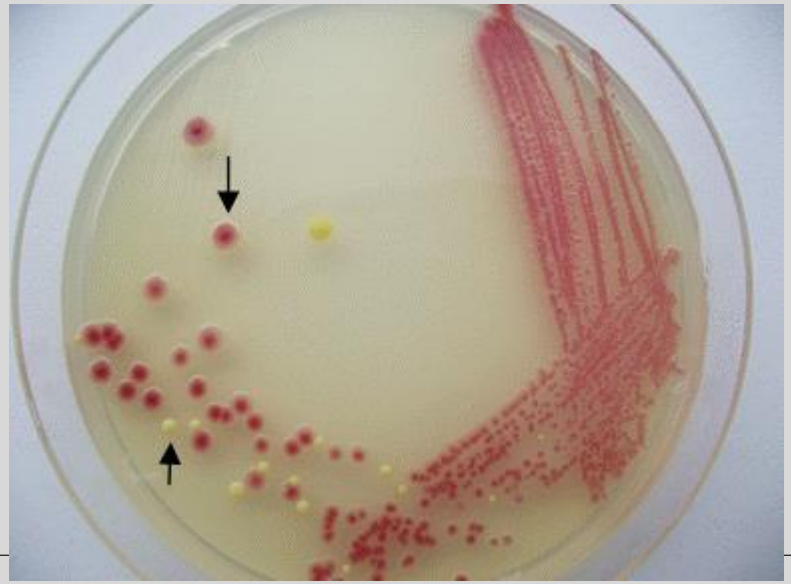
CLED . e-coli

Streaking plate method





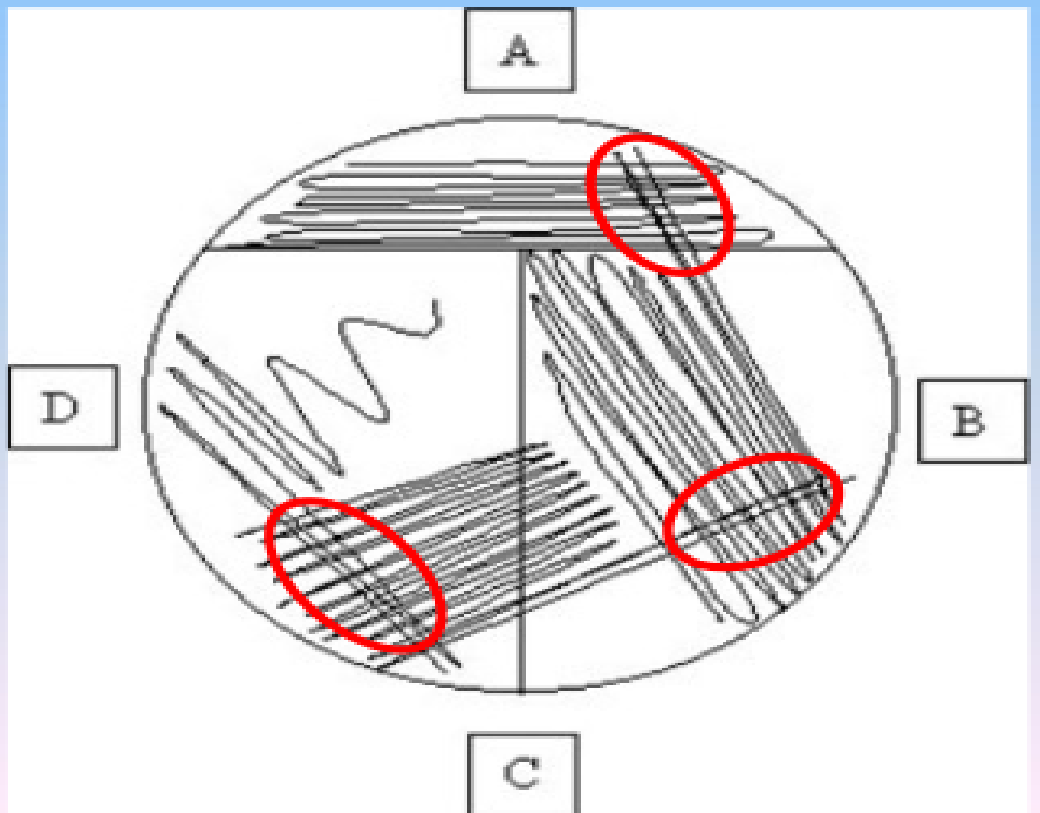




Streaking

Procedure:

1. Flame the loop and streak a loopful of broth culture as at A in the diagram.
2. Reflame the loop and cool it.
3. Streak as at B to spread the original inoculum over more of the agar.
4. Reflame the loop and cool it.
5. Streak as at C.
6. Reflame the loop and cool it.
7. Streak as at D.
8. Incubate the plate inverted.



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