Course: MBIO 240

Laboratory Skills

LECTURE 6

- POURING OF MEDIA
- ISOLATION OF FUNGI AND BACTERIA FROM DIFFERENT SOURCES

RECALL:

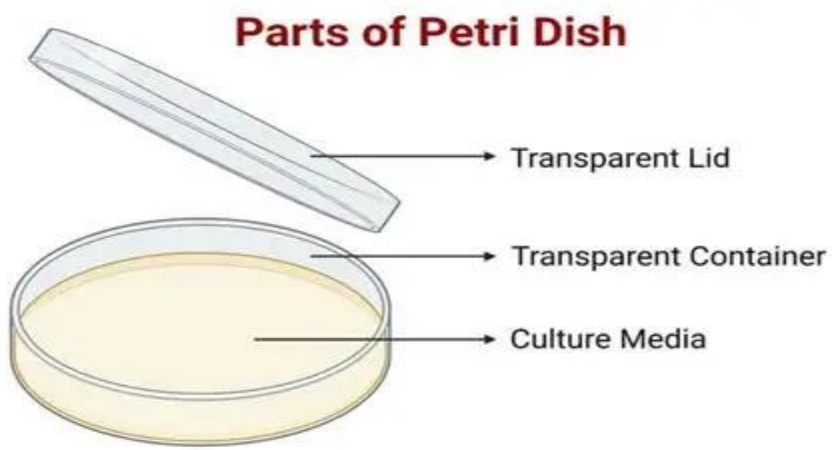
In the last lab, we learned about the preparation of two different media, namely Potato Dextrose Agar (for fungi) and Nutrient Agar (for bacteria), as well as their sterilization method (autoclaving).

Pouring of culture media

Aim of the experiment

❖To learn the pouring of sterilized media in petri dishes in aseptic conditions and isolate fungi (PDA) and bacteria (NA) from different sources.

- PDA-Potato dextrose agar
- NA-nutrient agar



Pouring of culture media

- Presently, clear plastic disposable petri dishes, typically 95 or 100 mm in diameter, 20 per sleeve (packet), are used in labs.

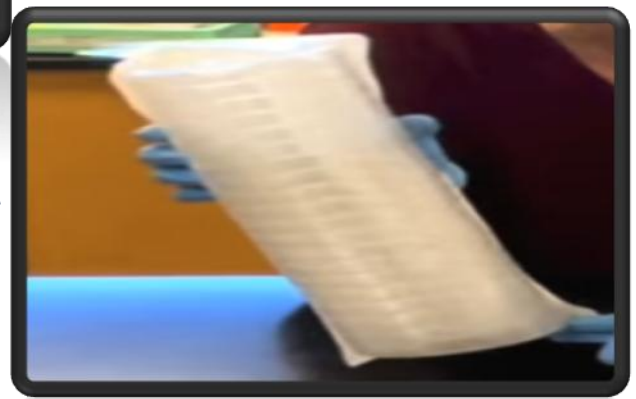
Steps :

- After autoclaving, pour the media into the plates using aseptic technique, preferably in a sterile cabinet

a) Sterilize the work space

Prepare a suitable work area by using a disinfectant. (Preferably, a laminar flow hood is used to avoid contamination).

Once the area is disinfected, place the petri dishes on the sterilized surface with their lids closed



Label the petri dishes

Labelling the petri plates on the bottom half with the following.

- Type of media used
- Initials
- Date
- Type of specimen

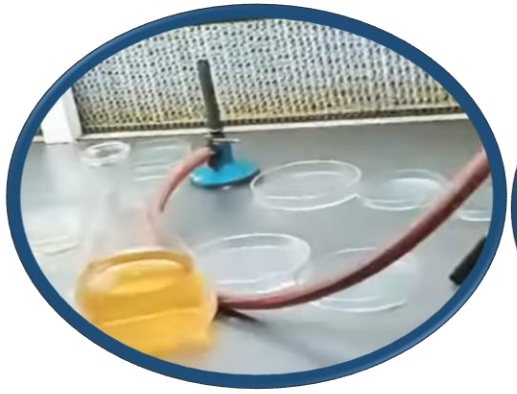
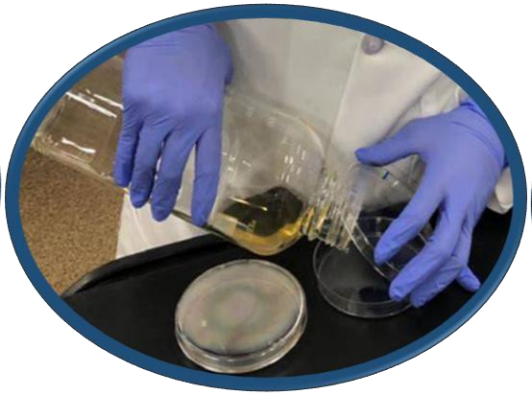
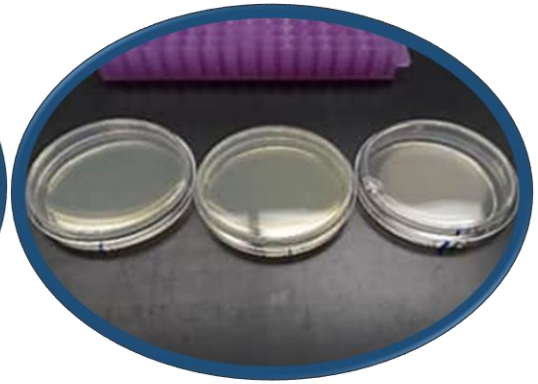
Pouring of culture media

Pouring

1.Remove the media from the autoclave, or if the media was already prepared, reheat it in hot water bath or a microwave until it melts.

2.Let the media cool, or carefully swirl until it is comfortable to the touch, but is still in a molten state.

- Pour aseptically into the base of the Petri plate (15-20 ml).
- Wait until solidified (15 -30 minutes).
- Store the solidified plates upside down in a refrigerator to avoid condensation

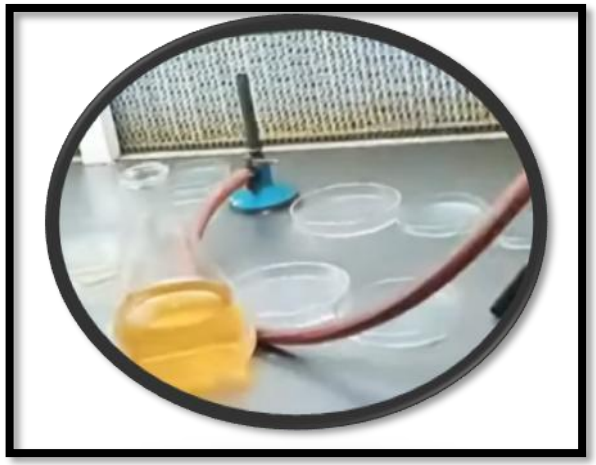






Pouring

Heat the media in water bath

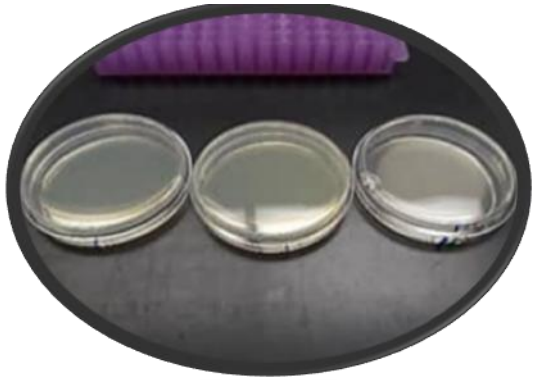
Or

Remove the media from autoclave

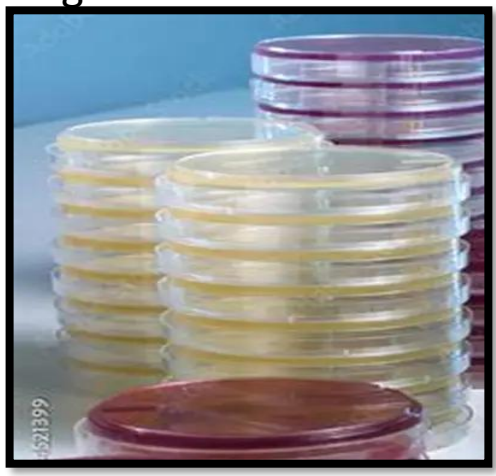


Disinfect the area and Prepare the petri plates for pouring

Pour the media in petri plates



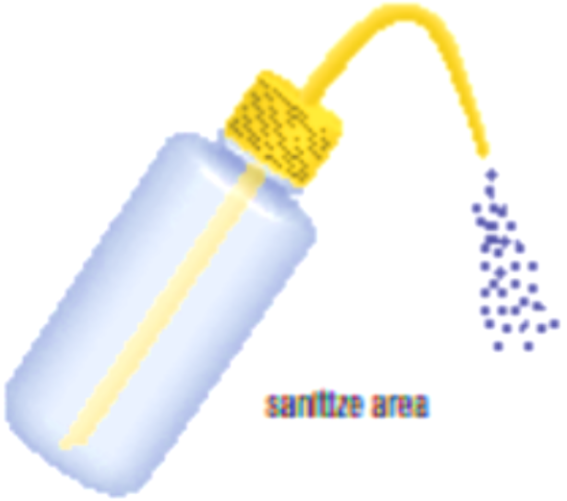
Allow it to cool and solidify



Place the solidified media in the Refrigerator in an upside-down manner to prevent condensation

Pouring of culture media

How to Pour Melted or Recently Autoclaved Media



1

Select a draft-free area to pour your plates.

Clean the area with 70% ethanol or a 10% bleach solution.

Wash and dry your hands.

2

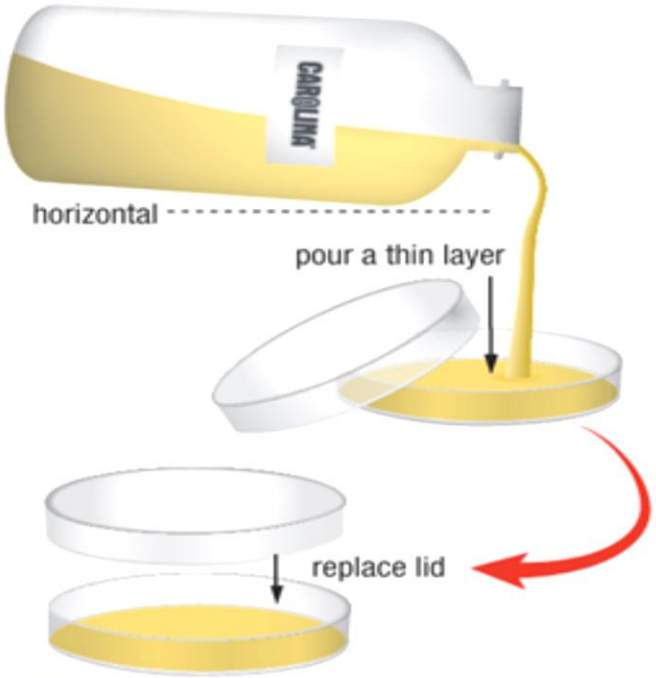
Set out closed sterile plates. Plan to pour all the media at once. Multiple pours increase the risk of contamination.

3

Cool the agar to 45° C. Sterilize the mouth of the bottle with a flame or an alcohol pad.

Once the bottle of media is cool enough (to about 45–55° C), the agar is ready to pour.

Pouring of culture media



4

Minimize contamination by keeping the bottle as close to horizontal as possible and lifting the lid of the dish just enough to pour the media.

Pour a thin layer of media, enough to cover the bottom of the plate, then immediately replace the lid.

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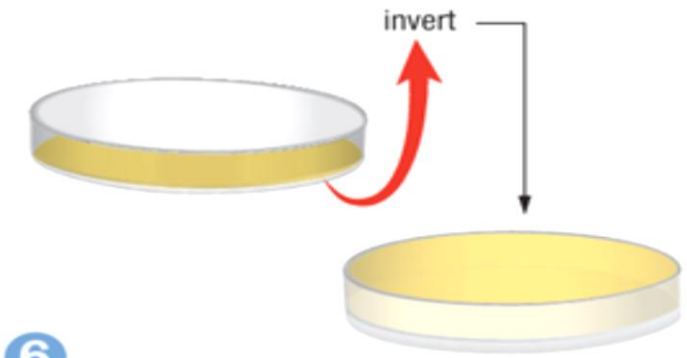
Allow at least 30 minutes and up to overnight for the media to solidify before using.



6

Invert the plates so that the media is on top and the lid on the bottom. This will minimize condensation on the plates.

Follow the link for video on pouring
https://youtu.be/vlNvX9o5SPM

7

Place inverted plates in a plastic bag, label with the type of media, and store at 4° C.

Plates may be stored for 2–4 weeks before use.

Some important points to remember while pouring media in a petri plate

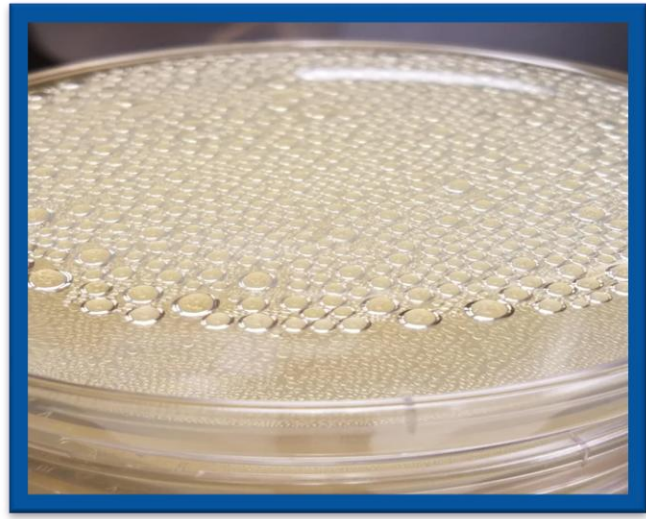
- You must work quickly, because once the agar container is below 45 °C, it will start to harden within 2-3 minutes.
- Use heat-resistant gloves
- When pouring into the petri dish, pour just enough (15-20 ml)to cover the bottom; this should fill the dish about half full (or half empty).
- Although you must work fairly quickly, pour the agar gently to minimize the number of bubbles
- **When you are ready to pour and have labelled dishes :**

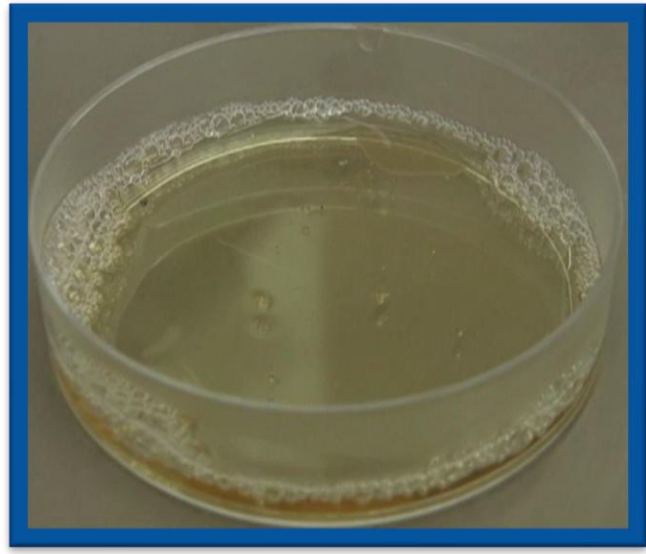
a) Open the cover of the petri dish halfway and keep the lid pointed down.

b) Pour the agar to just cover the bottom of the dish. Try to minimize the introduction of bubbles.

c) Repeat for all the dishes and keep them in stacks of five. Do not let the agar slosh up on the sides or lid.

d) Immediately rinse the flask with warm water to facilitate washing the flask.





Isolation of fungi

AIM

To isolate plant pathogenic fungi from infected plant materials.

PLANT PATHOGENIC FUNGI (PHYTOPATHOGENS)

- Plant Pathogenic fungi are a specific group of fungi that can invade plant tissues, causing various diseases.
- These pathogens are a significant concern as they cause considerable losses to crops worldwide.
- Fungi are considered among the dominant causal agents of plant diseases.

Why isolation?

Isolating these fungi is crucial :

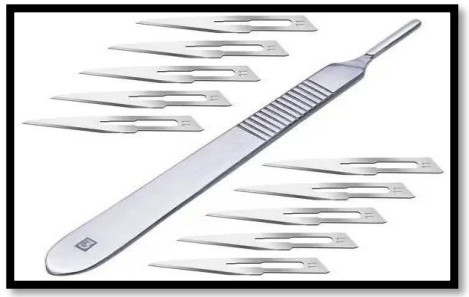
- **For studying their characteristics.**
- **Identifying the species involved.**
- **Understanding how they infect plants.**

➢The process typically involves culturing the fungi on a suitable medium, such as Potato Dextrose Agar (PDA), under controlled conditions to allow fungal growth and subsequent identification.

Note: Besides plants, fungi can infect humans, animals and birds. They are present in soil, dead decaying matter and in the air.

Materials and Methods

- Collect Infected plant material (fruit, vegetable or any plant part)
- Media: Potato Dextrose Agar (PDA) plates
- Conical flask
- Sterile distilled water
- 70% ethanol
- Teasing needle
- Scalpel
- Forceps
- Bunsen burner



Procedure

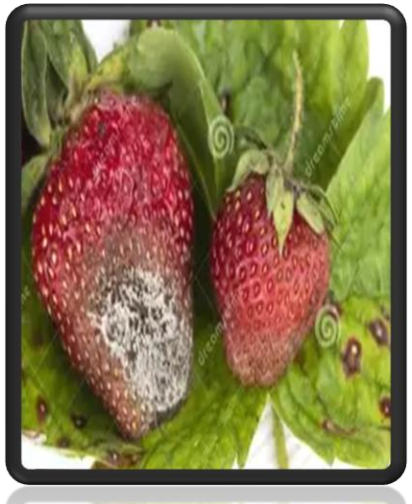
- Collect plant materials showing visible signs of infection, such as lesions or discolouration, that may indicate fungal infection.

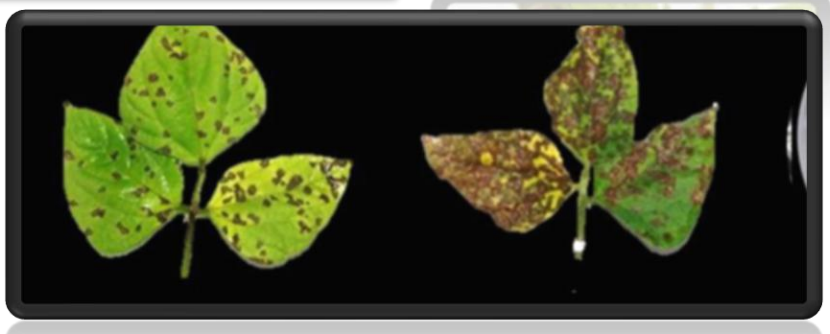
Procedure

- Disinfect the surface of the collected plant materials by dipping them in 70% ethanol for 1 minute.
- Wash the materials thoroughly with sterile distilled water to remove any residual ethanol.
- Under aseptic conditions, use a sterile scalpel or knife to cut the infected plant material into small pieces (approximately 2-3 mm).
- Aseptically transfer the small pieces of infected plant material onto the surface of Potato Dextrose Agar (PDA) plates.
- Incubate the PDA plates at 25-28°C for 5-7 days. - After 5 days, observe the growth of fungal colonies.

Procedure

➢Collect plant materials showing visible signs of infection and subject it to disinfectant to remove any external particles and then wash to remove the disinfectant.

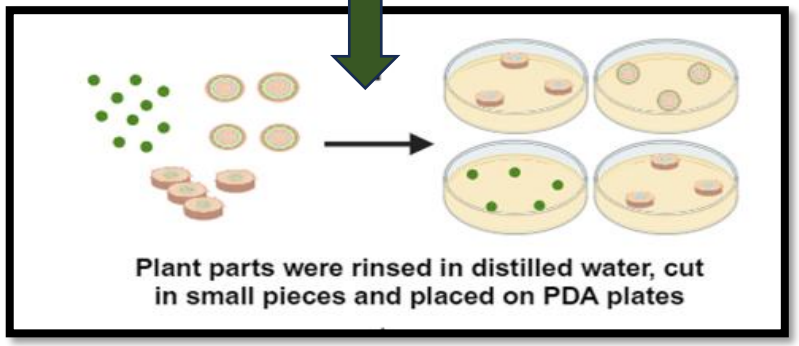




Cut the infected portion of the plant part and wash it with water

Dip them in 70% ethanol for 1 minute.

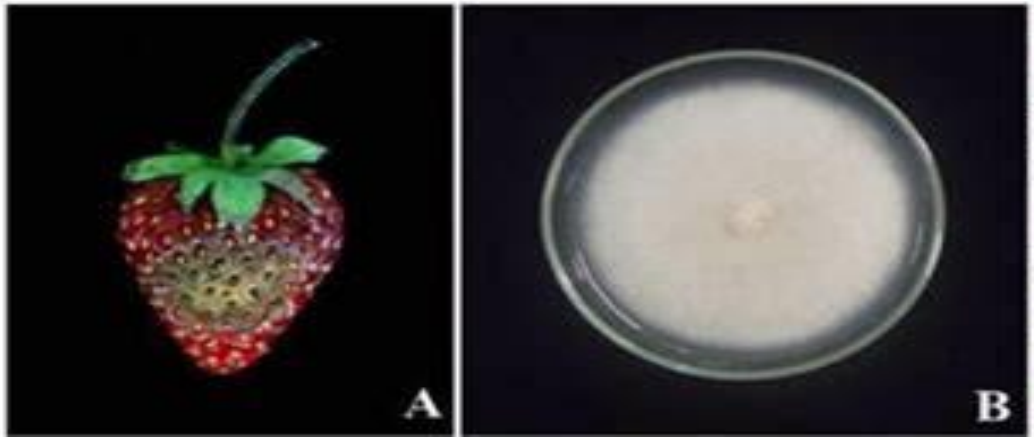
Wash with sterilized distilled water to remove excess ethanol



Incubate at 25-28°C for 5-7 days.

Results and Observation

Fungal growth should appear as distinct colonies on the PDA plates. The morphology of the colonies, such as color, shape, and texture, will be observed and used for preliminary identification.



fungal colony isolated from strawberry spots on a PDA plate

fungal colony isolated from the fungal spots of Spinach leaf on a PDA plate

Conclusion

- By following the procedure, plant pathogenic fungi can be successfully isolated from infected plant material.
- This process aids in the identification and study of fungal pathogens, which is essential for managing plant diseases.

Isolation of Bacteria from the Human Body

Aim of the Experiment:

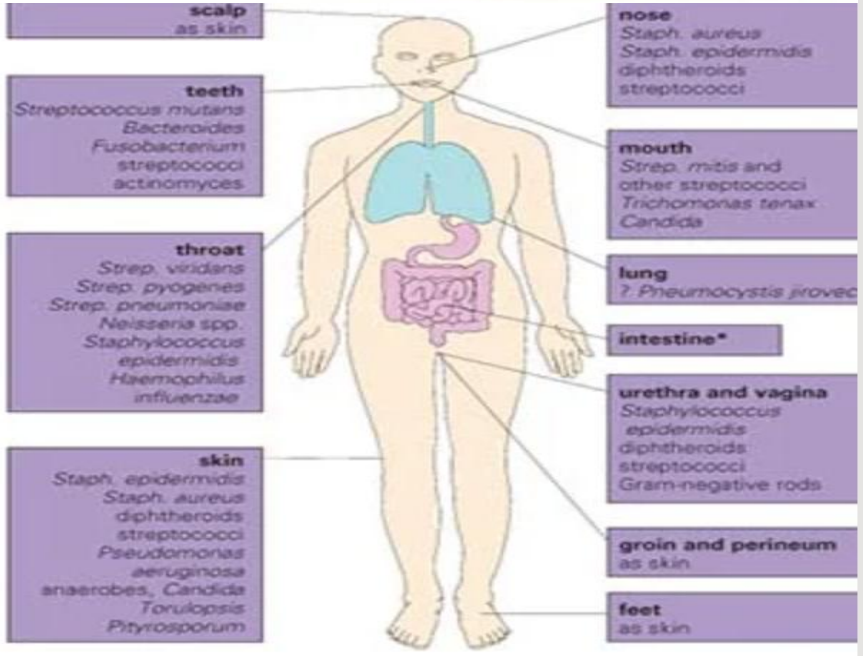
- To isolate bacteria from the human body to understand the normal flora.

What is a normal human flora

- The human microbiome refers to the collective sum of all microorganisms that reside on or within the human body.

- Bacteria are naturally present on various surfaces of the human body, forming a complex microbiome.

- These microorganisms play crucial roles in maintaining health but can also be involved in infect



Why isolate Bacteria from human body (normal flora)

The isolation of bacteria from human samples helps in the following:

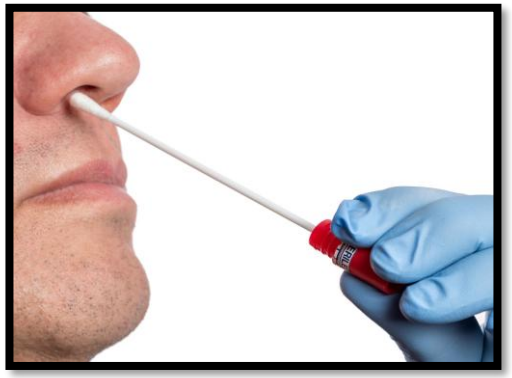
- Diagnosing infections
- Understanding the composition of the normal microbial flora.

This procedure involves:

- Collecting samples from the body
- Inoculating them onto suitable growth media
- Incubating the cultures to allow bacterial growth.

Materials, Methods & Equipment:

- Sterile swabs
- Nutrient agar plates
- Nutrient broth
- Inoculating loop
- Incubator
- Bunsen burner



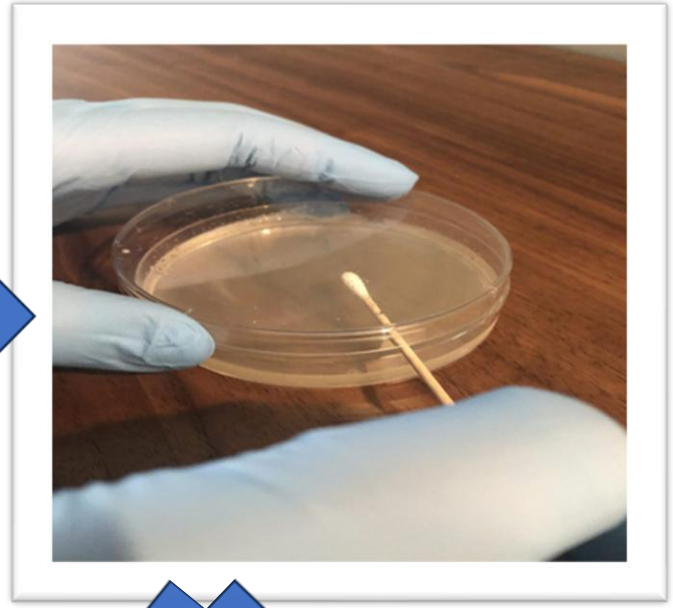
Materials, Methods & Equipment:

- Collect a sample using a **sterile cotton swab moistened in sterile saline.**
- Obtain a specimen from the skin by rubbing the swab against the palm of the hand or from the appropriate body site (e.g., nose, skin, ear, or throat).
- Transfer the sample on the swab into sterile NA agar, and spread the sample by slightly rubbing the swab on the agar surface of the plate and incubate at 37°C for 24 hrs.
- After incubation of plates at **37°C for 24 hours**, examine the nutrient agar plates for bacterial growth
- Select isolated colonies for further identification, and perform the Gram staining technique to differentiate between **Gram-positive and Gram-negative bacteria**

Materials, Methods & Equipment:

Sterile moist swab

Sample collection from various parts of the body

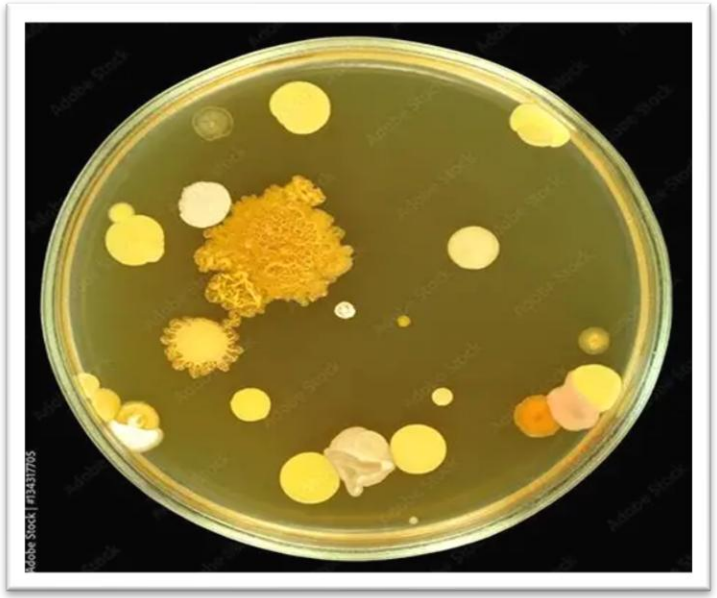


Transfer the bacterial sample to the agar surface

Bacterial Colonies isolated from the samples

Results & observations:

- Bacterial growth should appear as distinct colonies on the nutrient agar plates.
- The colonies may vary in color, size, and shape depending on the bacterial species.
- Gram staining of isolated colonies will provide further details on the bacterial classification.



Conclusion

By isolating bacteria from human body samples, the experiment allows for the identification and study of normal microbial flora. This is important for understanding the roles of these microorganisms in health and disease