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جامعة المستقبل
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Advanced Laboratory Techniques

Third class

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Lecture / 5

Culture Media / part 2

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There are some common types of culture media and their uses:

1. **Nutrient Agar**: Nutrient agar is a general-purpose, non-selective medium that supports the growth of a wide range of microorganisms. It contains nutrients like peptones, beef extract, and agar, which provide essential elements for bacterial growth.
2. **Blood Agar**: contains blood (usually from sheep) and nutrient agar. It is used to culture a variety of bacteria, particularly those that require additional nutrients, such as hemolysis testing for *Streptococcus* species.
3. **MacConkey Agar**: is selective for Gram-negative bacteria and differential based on lactose fermentation. It contains bile salts and crystal violet to inhibit the growth of Gram-positive bacteria and indicators that change color in response to lactose fermentation.
4. **Sabouraud Agar**: Sabouraud agar is used for the isolation and cultivation of fungi. It contains peptones and dextrose and is acidic, which inhibits the growth of bacteria while allowing fungal growth.
5. **Mannitol Salt Agar**: is both selective and differential. It contains high salt concentration to select for halophilic organisms, and it differentiates *Staphylococcus* species based on their ability to ferment Mannitol.
6. **EMB Agar (Eosin Methylene Blue Agar)**: is selective for Gram-negative bacteria and differential based on lactose fermentation. It contains eosin and methylene blue dyes that inhibit the growth of Gram-positives and differentiate between lactose fermenters and non-fermenters.
7. **Thioglycolate Broth**: is a liquid medium used for the cultivation of anaerobic bacteria. It contains thioglycolate to reduce oxygen levels in the medium.
8. **Lowenstein-Jensen Medium**: This medium is used for the culture of *Mycobacterium* species, including the bacteria that cause tuberculosis. It contains egg-based components and malachite green to inhibit the growth of other microorganisms.
9. **TSA (Tryptic Soy Agar)**: TSA is a general-purpose medium that supports the growth of a wide range of microorganisms. It's used for routine laboratory purposes and in the quality control of commercial products.
10. **Brain heart infusion broth-glycerol medium:** It was used for preservation of bacterial isolates as stock for long.

11. **Brain heart infusion (BHI) broth:** used as transported media transfer the samples to the laboratory.
12. **MR-VP medium**: used to detect the partial and complete hydrolysis of glucose
13. **Muller-Hinton agar medium:** Muller-Hinton agar medium used in anti-microbial susceptibility testing.
14. **Kligler iron agar medium:** used for determining glucose and lactose fermenter isolates and possible hydrogen sulfide (H_2S) production as a first step in the identification of gram negative bacilli.
15. **Simmons' citrate medium:** used for determining the ability of bacteria to utilize citrate as the sole carbon source.
16. **Urea agar medium:** This medium was used to test the ability of bacteria to produce urease enzyme.
17. **Motility medium**: This medium was used to detect bacterial motility.
18. **Luria broth medium:** used to DNA extraction.

Methods of culture

The plate streak method

- The plate streak method is a microbiological technique used to isolate individual bacterial colonies from a mixed population.
- This technique is commonly used in clinical and research laboratories for microbial identification and analysis.

There are the steps for performing the plate streak method for colony isolation

Materials and Equipment:

1. Sterile agar plates (Petri dishes) containing a suitable agar medium. Inoculating loop or sterile bacteriological loop. Sample containing the mixed bacterial population.
2. Incubator set to the appropriate temperature and atmosphere for the bacteria being isolated. Marker for labeling the plates.

Procedure:

1. Select the appropriate agar medium based on the requirements of the experiment or the type of bacteria you want to isolate.
2. Label the bottom of the agar plate with relevant information, including date, sample source, and any other necessary details.

3. If using an inoculating loop, sterilize it by passing it through a flame until it glows red. Allow it to cool briefly, so it does not harm the bacteria during streaking.
4. **First Streak;** open the agar plate carefully and hold the lid in one hand or place it upside down to minimize contamination.
5. Using the sterilized loop, take a small sample from the mixed population (e.g., a mixed culture from a broth or a sample from a swab).
6. Streak the loop across the first quadrant of the agar plate's surface. Be sure to streak in a way that spreads the sample thinly and evenly in that quadrant.
7. **Second Streak (T- or Zigzag Streak);** sterilize the loop again. Streak the loop from the first quadrant into the second quadrant by passing through the first quadrant several times. This further dilutes the bacteria. Avoid touching the areas already streaked in the first quadrant.
8. **Third Streak (Inverted Triangle or Zigzag);** Sterilize the loop again. Streak from the second quadrant into the third quadrant, further diluting the bacteria and isolating individual colonies. Avoid crossing into the previously streaked areas.
9. **Fourth Streak (Single Colonies);** Sterilize the loop again. Streak from the third quadrant into the fourth quadrant in a way that ideally results in well-separated individual colonies. Ensure the loop does not pass back into any previously streaked regions.
10. Close the agar plate and incubate it at the appropriate temperature and atmosphere for the bacteria the want to isolate (e.g., 37°C for most common bacteria). Incubate for the required time (usually 24-48 hours) until colonies grow and can be observed.
11. After incubation, observe the plate for the growth of individual colonies.
12. Analyze the colonies' characteristics, such as size, shape, color, and texture, as these can provide information for bacterial identification.

The stabbing method

- Also known as the deeq1 1p stab or deep inoculation technique, is a microbiological procedure used for culturing anaerobic bacteria or microorganisms that thrive in the absence of oxygen.
- This method involves inoculating a solid agar medium inside a tube or container by introducing microorganisms deep within the medium.

Materials Needed:

- Sterile agar medium in a tube (commonly used for this method is a deep agar tube)
- Bacterial culture or sample, Inoculating needle or wire.

Procedure:

- Select an appropriate agar medium, such as a deep agar tube containing a solidified agar medium. Ensure that the medium is appropriately prepared and solidified.

- Before starting, label the tube with the necessary information, including the date, sample source, and any relevant identifiers.
- The inoculating needle or wire should be sterilized by passing it through a flame until it becomes red-hot. This process ensures that it is free from contaminants.
- Allow the inoculating needle/wire to cool for a few seconds, so it's not too hot.
- Using aseptic technique, dip the cooled inoculating needle/wire into the bacterial culture or sample.
- Open the tube containing the solidified agar medium. Hold the tube at an angle to create a small opening in the medium.
- Insert the inoculating needle/wire deep into the agar, ensuring it reaches the bottom of the tube. This ensures that the microorganisms are introduced deep within the medium.
- Carefully close the tube to prevent contamination from the surrounding environment.
- Incubate the inoculated agar medium tube at the appropriate temperature and conditions required for the specific microorganism being cultured. For anaerobic organisms, incubation is typically done in an anaerobic chamber or using specialized anaerobic conditions.
- After incubation, examine the tube for the growth of the microorganisms. Anaerobic bacteria will typically grow deep within the agar medium, away from the oxygen at the surface.

The stabbing method is valuable for culturing anaerobic bacteria and other microorganisms that cannot tolerate oxygen. It allows for the growth and isolation of these organisms in an environment suitable for their needs.

Steps of antibiotic susceptibility test

- Ii is a crucial step in identifying effective antibiotics for treating bacterial infections.
- The procedure for antibiotic susceptibility testing using the disk diffusion method, a widely used technique in clinical microbiology.

Materials and Equipment

1. Obtain a pure culture of the bacteria that want to test.
2. Prepare and sterilize Mueller-Hinton agar plates. This medium is commonly used for antibiotic susceptibility testing.
3. Acquire commercially available antibiotic disks. These disks contain a specific concentration of antibiotics.
4. For inoculating bacterial suspension onto the agar plates.
5. Set at the appropriate temperature for the bacteria being tested.

Procedure:

1. Prepare a bacterial suspension in sterile saline.
2. Using a sterile swab or loop, streak the bacterial suspension evenly over the entire surface of the Mueller-Hinton agar plate.

3. Place the antibiotic disks onto the inoculated agar surface. Ensure that the disks adhere well to the agar.
4. Incubate the plates at the appropriate temperature for the bacterium being tested (e.g., 35-37°C for most common clinical isolates). Incubation time can vary but is typically 16-18 hours.
5. After incubation, measure the zones of inhibition around each antibiotic disk. The diameter of the zone is inversely proportional to the resistance of the bacteria to the antibiotic.
6. Compare the zone diameters to standardized tables or guidelines provided by organizations such as the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST).
7. Report the results as susceptible, intermediate, or resistant based on the established breakpoints.