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BASIC MICROBIOLOGY PRACTICAL FOR PHARMACY STUDENT

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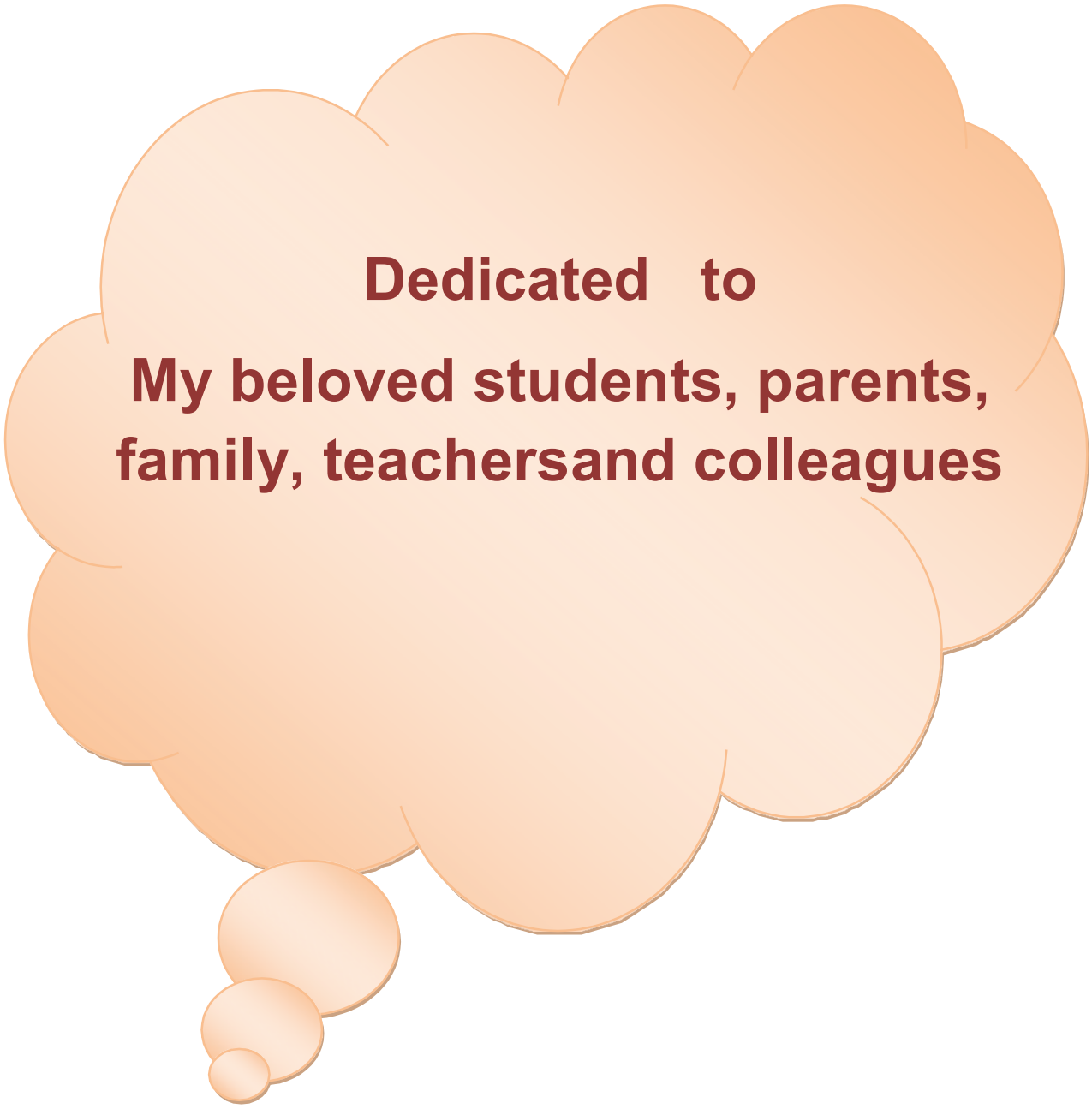

Dedicated to
My beloved students, parents, family, teachersand colleagues

BASIC MICROBIOLOGY PRACTICAL FOR PHARMACY STUDENT

Preface

It gives us huge pleasure to place before the B.pharm pharmacy students the book on Practicalbook on pharmaceutical microbiology.

This book has been written strictly in unity with the current syllabus prescribed by pharmacycouncil of India for B.pharm students.

Keeping in view the requirements of syllabus and teachers, this book has been written to coverthe entire practical in an easy manner.

All efforts have been made to keep the text error –free.

However any suggestions and constructive comments would be highly appreciated and incorporated in the future edition.

ACKNOWLEDGEMENTS

I would like to acknowledge my indebtedness and render my warmest thanks to all my seniorsand invaluable help rendered by my students.

Dr. Juhi Singh

CONTENT

EXPERIMENT NO. :-1

EQUIPMENT COMMONLY USED IN MICROBIOLOGY LABORATORY:

- Autoclave
- Hot air oven

Incubator Sterilization can be achieved in the following ways:

- By steam (moist heat)
- By extended heating in an oven (dry heat)
- By complete burning, i.e. incineration moist heat speedily kills viruses, bacteria and fungi. Exposure to boiling water for 10 minutes is adequate to destroy vegetative cells. On the other hand, this temperature isn't high sufficient to destroy bacterial endospores and may stay alive even after many hours of boiling. As a result, boiling may be used for killing cells in drinking water but it doesn't sterilize entirely.

AUTOCLAVE:

PRINCIPLE: The temperature of saturated steam at standard atmosphere pressure is 100°C. The compressed steam or steam under pressure is conducting higher temperature (hotter) than boiling water. As given away in tyndallization, higher the steam pressure, higher shall be the temperature. So, when water is positioned in a closed vessel like an autoclave or a pressure cooker, the temperature of water will be 100°C.

The air present in the autoclave shall adversely affect saturation of steam inside the apparatus. So, all the air that surrounds and covers the lid must be detached just before we start on actual steam sterilization. The moist heat kills microorganisms by denaturing and coagulating bacterial enzymes and natural proteins. It may also interrupt the cell membrane.

TYPES:

There are two types of autoclaves –

AUTOCLAVE

Pressure cooker type Gravity displacement model.

WORKING:

- First Autoclave and the material to be sterilized load and close the door.
- Release that air outlet valve to permit the air in the chamber to be displacing by the arrivingsteam.
- Open that steam supply valve so that steam can be admit to the sterilizing chamber.
- From steam line jacket, regularly watch the temperature gauge as it approaches at 100°C.
- At the temperature, air present in the chamber will elevate the pressure for routine withsterilization set for temperature at 15 PSI.

- Note down the time for sterilization.
- If the air isn't completely removed, it will show less temperature than 121°C.
- Later than about 15-20 minutes, close up the autoclave by closing the steam valve supply.
- Wait until the pressure falls to zero on the gauge.
  - The pressure will fall slowly. Open the door of the autoclave gradually when pressure gauge shows zero, indicating no steam pressure.
  - During intervals, it is essential to ensure the efficiency of sterilization. That means we must ensure whether the autoclave is working or not.
- It forms a significant port control program for many industries and work articles.
  - The substances which are more usually sterilized by autoclave are most used for biological media, e.g., aqueous solution containing alcohol, volatile oils and substances like sugar, surgical dressing, glassware, Petri dishes, fillers, pipettes, dry heat sensible materials approximating rubber, plastic items etc.

HOT AIR OVEN:

PRINCIPLE: The propose of an oven for the sterilization of pharmaceutical products mustsatisfy the following requirements:

- Preference of suitable temperature.
- Every item inside must receive correct exposure, wherever it is located.
- The sterilizing temperature has to be reach quickly and maintain with small variation.
  - These aims are nearly fulfilled by electrically heated thermo statistically (control oven fancirculation.)

An oven consists of:

- An aluminum and stainless steel chamber, divided from the outer case by a thick layer of glassfiber insulation.
- The hollow flanged door is also filled filling and carries on asbestos gasket then provided tightseal.
- Heater can be located in the side walls as well as under the floor, giving the best distribution ofheat.

- Heat is conferring by conduction, convection and radiation.
- Perforated shelves (3 to 4) are provided in the chamber, which are detachable.
- Good quality thermometer is packed in the front of the chamber and a vent is filled at the top ofthe oven.
- The oven is fixed which a fan is fitted in the back wall for air circulation.

HOT AIR OVEN

WORKING: The oven should be brought up to operating temperature before the load isplaced in it.

LOADING:

- For the period of cooling, non-sterile air is drawn in so it may contaminate the articles. Thearticles placed in oven must be plugged and wrapped.
- For getting good results, the oven should be properly loaded, such that each can take deliveryof the maximum radiation through the walls.
- The vent should be closed.
- The door can then be shut and fan switched on.

- While oven reaches sterilization temperature, heating is continued for long and high exposuretime.
- After switching off, the door is left closed until the temperature has reached 40°C.

ADVANTAGES:

- It is useful for substances scratched by moisture, e.g., oils and powder.
- It is useful for assembled equipment as there is adequate time for penetration.
- A smaller amount destructive to glass and metal equipment than soggy heat.

DISADVANTAGES:

- Provide severe situation, such as high temperature and elongated exposure to most medicaments, rubber and plastics and thus, cannot be sterilized.
- Preparation containing water, alcohol or volatile liquids can't be sterilized as they mightevaporate at high temperature.
- Unsuitable for surgical dressings.

APPLICATIONS: Glassware, anhydrous powder, equipment, oils, absorbable powder.

INCUBATOR:

Incubator is the cabinet in which only preferred temperature may be constantly maintained.

PRINCIPLE:

The majority of the modern incubators are heated electrically which the top of heaters are provided at the bottom of the cabinet. As air at the bottom is heated, it gets lighter and rises up while cold air is pressed down at the bottom. This generates convection current for air circulation. Cycle continues until desired temperature is reached, which is normally the opium temperature of most organisms.

CONSTRUCTION:

Cabinets are made up of non-corrosive metals which are lying inside with Asbestos to avoid loss of heat. The metallic door opens at front. At the back it, another glass door is provided to watch the temperature without opening the door. Thus, loss of radiation is minimized. The cabinet is provided with a temperature control system which is restricted by resulting nodes on sides. Plot temperature will light when heating is continued and will be off when cabinet is heated to the earlier set temperature. Temperature can be read by inserting a thermometer from the hole provided at the top of the cabinet. Incubators have a wide temperature range varying from a little over the temperature at room to more than 60°C.

USES:

Incubators are used for providing constant temperature for the growth of microorganisms so that cultivation is carried out at an optimum temperature. Normally, the temperature of incubator is constantly maintained at 37°C.

B.O.D. Incubator

EXPERIMENT NO. : - 2

AIM- STERILIZATION OF GLASSWARE, PREPARATION AND STERILIZATION OFMEDIA**.**

REQUIREMENTS- Petri plate, Loop (wire/plastic), Spreader (glass/plastic), Forceps (metal/plastic), Straight wire, Pipette(calibrated/dropping; glass/plastic), Conical flask, Autoclave,/roasting bag, Hot air oven, Microwave oven, Incubator, Test tube, Water bath, Thermometer, PH meter, Cupboard, Refrigerator, Microscope, slides, covers slips, Stains, staining rack, immersion oil.

THEORY –

Method of sterilization–

Sterilization is utilized for a physical or chemical procedure to demolish all microbial life, including highly resistant bacteria spores. This can be done either by make use of dry heat or by moist heat.

DRY HEAT:

Flaming - Inoculating loops or wires, point of forceps and searing spatulas are seized in a Bunsen flame turn over they turn out to be red hot, for sterilizing them. If the loops hold infective proteinaceous material, they should be first curved in chemical disinfectants before flaming to prevent spattering. Scalpels, cover slips, mouths of culture tubes, needles, glass slides,etc. could be approved a few times through the Bunsen flame without allowing them to become red hot. The bacteria get damaged needles, basins and scalpels are from time to time immersed inmethylated spirit and the spirit burnt off them.

ii) **Hot air oven:** This is the most widely used method of sterilization by dry heat at 160°C - 170°C for 2-4 hours in a hot air oven. It is used to sterilize glassware like test tubes, conical flask, beakers, Petri dishes, measuring cylinders etc; forceps, scissors, syringes, scalpel, swabs, some pharmaceutical products such as liquid paraffin, dusting powder etc. Hot air is bad conductor of heat and its stabbing power is low. The oven is usually heated by electricity, with heating elements in the wall of the cavity and it must be fitted with a fan to ensure even distribution of air and elimination of air pockets. It should not be overfull. Dry heat sterilizers should be monitored on a standard basis using proper indicators.

Rubber materials except for silicon rubber- will not stand the temperature. The material should be prearranged in a manner, which allows free circulation of air in between. Glassware should be entirely washed and dried before being placed in the oven. Test tube, flask, measuring cylinder etc. should be plugged with cotton wool and covered with foil.

Supplementary glassware such as Petri dishes and pipettes should be wrapped in Kraft paper. A weighty duty foil pouch can be used to wrap glass pipettes. Glassware can be positioned in metal tray and finally in the hot air oven all these can be heated at 160°C - 170°C for 2-4 hours. These are then detached from the oven and cooled. Paper or cotton should not be kept back directly in the oven as these may be overdone. Plastic material such as pipette, plastic basket or trays to gripglassware should not be positioned in the oven.

iii) Incineration: This is an outstanding method for rapidly destroying materials such as

Coiled dressings, animal carcass, bedding and pathological material. Plastics such as polyvinyl chloride (PVC) and polythene can be dealt with similarly but polystyrene materials give out clouds of dense black smoke and hence should be autoclaved.

1. Petri dishes
2. Pipette - glass and plastic
3. Test tubes
4. Forceps

5. Scissors
6. Culture Media
7. Apron
8. Test tube rack (plastic)
9. Cotton swabs
10. Glass measuring cylinder.

PROCEDURE:-

Sterilization of glassware's:-

Wire loop: heat up to redness in Bunsen burner flame.Empty glassware and glass pipettes and

Petri dishes

Either: hot air oven, wrapped in either greaseproof paper or aluminum foil and held at 160 °C for

2 hours, allowing additional time for items to come to temperature (and cool down!). Or autoclave

Culture media and solutions: AutoclaveGlass spreaders and metal forceps Flaming in alcohol

(70 % IDA).

Preparation of culture media

1. Correctly weighed the constituents or ingredients of that exacting medium.

2. The necessary amount of water is calculated and poured into stainless steel pan or hot resistant beaker and the weighed ingredients are putted one by one into it. Liquid growth media containing nutrients are frequently solidified by the addition of agar. Agar - agar is a complex polysaccharide containing 3,6-anhydro-L galactose and D-galacto –pyranose , free of nitrogen , produced from various red algae belonging to geladium , gracilaria and other genera (Heating – 96 degree C , cooling – 40-45 degree C). Agar can be replaced with gelatin (10-16%w/v).

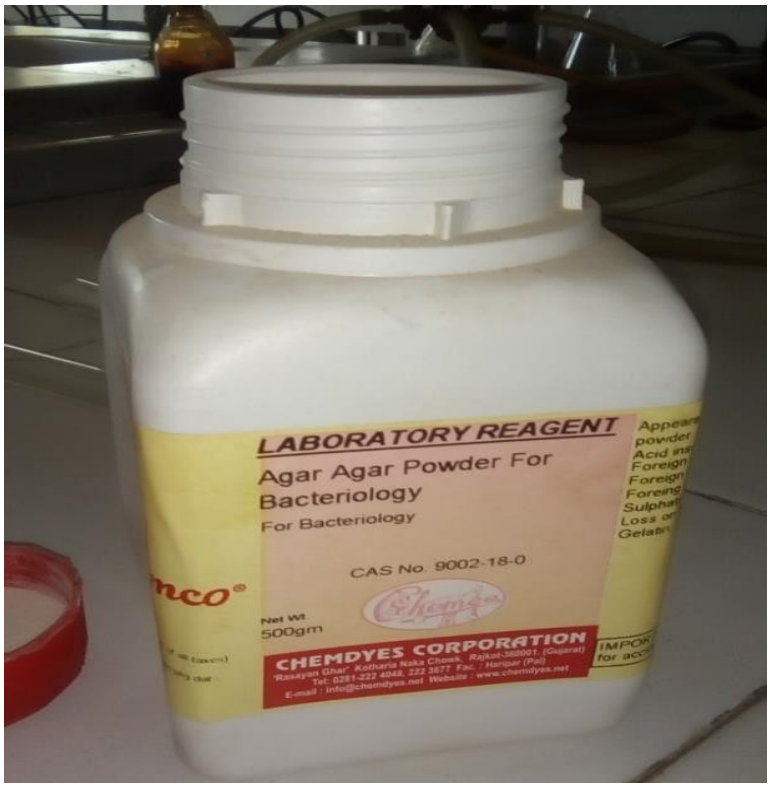
The agar is dissolved at last in case of agar based medium. To prevent the development of clumps, agar is dissolved separately in a small amount of water, which is then added to the rest of the solution, and the volume is brought to desired level by addition of distilled water.

3. When preparation is cooled pH is checked and if necessary pH is attuned.

4. The medium is now poured into test tube (15ml) usually in 10ml aliquots, in 500ml Erlenmeyer flasks of other containers. While in liquefied state, solid media can be added in the test tubes, which are ether allowable to cool and harden in a slanted situation producing agar slants or permitted to harden in the upright situation produce agar deep tubes. The space betweenagar surface and the neck of test tube or bottle should be at least 5cm.

5. The container is now closed with cotton plugs.

6. The medium now sterilized in an autoclave usually at 121 degree C for 15 minutes and allowed to cool to be used for the purpose.



POURING A PLATE

1. Assemble one bottle of sterile molten agar from the water bath.

2. Grasp the bottle in the right hand; take out the cap with the little finger of the left hand.

3. Flame the neck of the bottle.

4. Lift the lid of the Petri dish a little with the left hand and pour the sterile molten agar into thePetri dish and put back the lid.

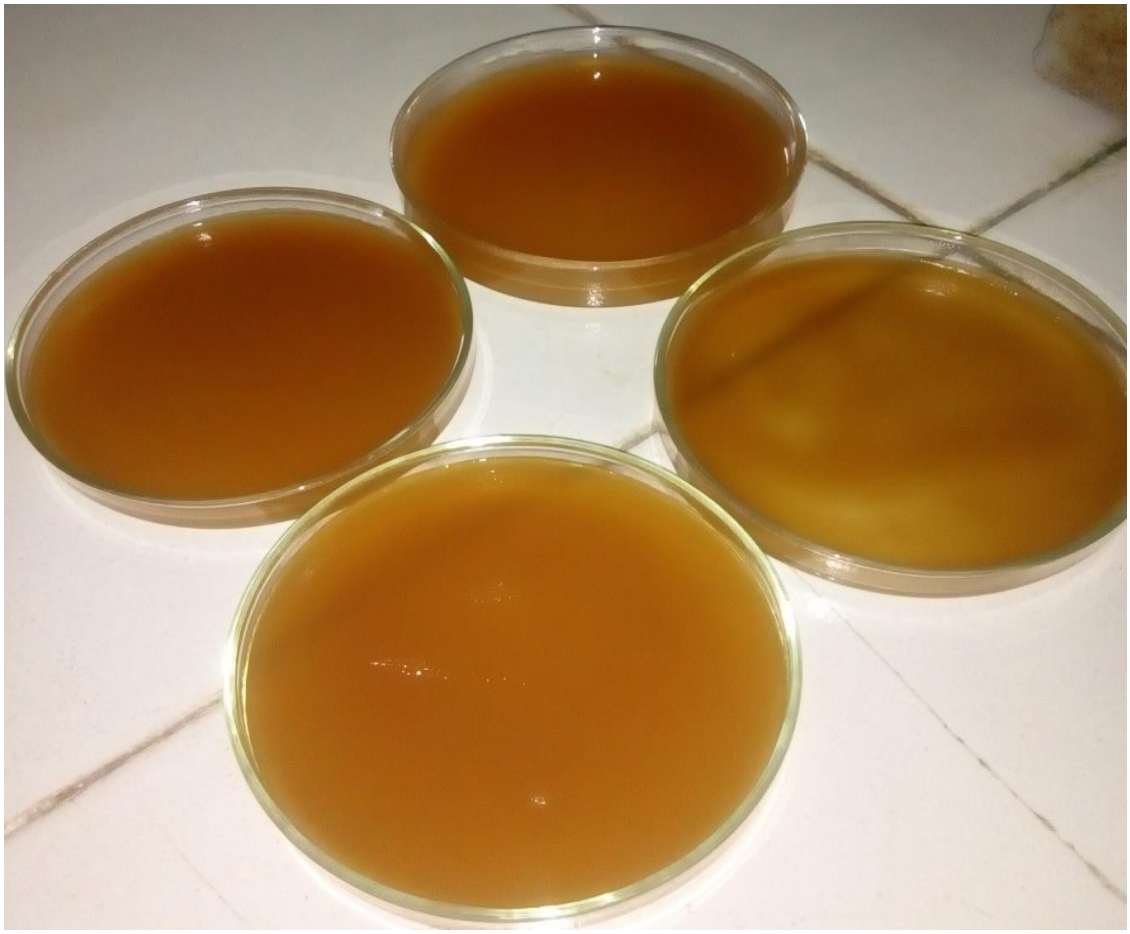
5. Flame the neck of the bottle and restore the cap.

6. Gently rotate the dish to ensure that the medium covers the plate consistently.

7. Permit the plate to solidify.

- The base of the plate must be sheltered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles.

-The plates should be used as rapidly as possible after pouring. If they are not going to be used directly away they need to be stored inside sealed plastic bags to prevent the agar from drying out.



Storage of media:-

Store stocks of prepared media at room temperature away from direct sunlight; a cupboard is ideal but an open shelf is acceptable. Media in vessels clogged by cotton wool plugs/plastic caps that are store for future use will be subject matter to evaporation at room temperature; avoid wastage by means of screw cap bottles. Re-melt store agar media in a boiling water bath,

pressure cooker or microwave oven. Various time ago melted, agar can be reserved molten in a water bath at ca 50°C until it is ready to be used. Sterile agar plates can be pre-poured and store in well-sealed plastic baggage.

Result: - All glassware's are sterilized and culture media is prepared and sterilized.

EXPERIMENT NO. : 3

AIM: SUB CULTURING OF BACTERIA AND FUNGUS

THEORY: Sub culturing is a technique of transfer microorganism from one container to a different, donation them with fresh supply of nutrient either in solid in liquid medium. The essential objective of this test is to prepare subculture of bacteria or fungus.

MATERIALS: Bacterial culture in nutrient agar plate, sterile medium, fungal culture in potato dextrose agar plate, Nutrient agar plate potato dextrose agar.

REQUIREMENT: Inoculating loop, Bunsen burner.

PROCEDURE: Sub culturing method of bacteria:

In a laminar air flow we get the culture plate containing the isolated colonies of bacteria grown on them. Then we obtain a or pick an isolated colonies or more colonies on the inoculating loop and then we close up this plat now we move a single colonies or a few colonies of bacteria into the new plate by streaking and zigzag method . To keep away from the contamination it is desirable to lift the lid of the plate of minimum. Then close the lid and incubate the plate for 48 hours

Sub culturing method of fungus:

In the laminar air flow we get the plate cultures containing fungus. Then we release very less as the spores may come out. Then the tip of inoculating loop in the Bunsen burner to sterilize it

.Then we acquire the loop into the culture and take the fungal culture and streak them into the new sterile PDA plate .Then we close the plate with the lid and inoculate it for 72 hrs.

Nutrient slab

Material Required Reagents: Beef extract (lab lemco), peptone, NaCl2, distilled water, agar Equipment and glassware: Pipettes with pipette aids, Pipette and Petri dish containers, autoclave,sterilized conical flasks, test tubes, screw cap tubes.

PROCEDURE

1. Weigh up the ingredients and dissolve in 800ml of distilled water, by gentle heating.
2. Cool the stuffing and adjust the pH to the preferred level.
3. Pass through a filter the contents through non-absorbent cotton or muslin cloth and regulatethe volume to 1000ml.
4. Distribute into sterilized tubes and flasks. Plug up them tightly with non-absorbent cotton andautoclave at 15psi for 15-20 minutes.

Slants preparation

Material Required Reagents: Sterile Nutrient agar Equipment and glassware: Petri dishes/plates- glass or plastic (at least 15 x 90 mm), screw cap tubes, cotton plugs, laminar air flow chamber, autoclave.

PROCEDURE

1. All the glassware sterilized by oven drying method i.e. 160^{0C} for 2hrs.
2. 2-3ml of nutrient agar taken in screw cap test tubes in non-sterile form.
3. These test tubes autoclaved at 121^{0C} for 15min.
4. For slant preparation few test tubes inclined and permissible to solidify.
5. For stab preparation the test tubes containing sterilized agar allowed to stand in an uprightposition and agar allowed to solidify at the base.

6. For pour plate preparation, after the addition of dilute sample, 15-18 ml of molten agar added to Petri plate. The stuffing mixed by rotating in clockwise and anticlockwise direction. The Petri plates then allowed cooling for solidification of agar. In a similar style, plates can be prepared forstreaking and spread plate however there is no addition of sample prior to pouring agar. The sample is loaded on the surface of Petri plate either with glass rod bent at 90^{o} or an inoculating.

CONCLUSION:

After following these methods we can easily sub culturing of bacteria and fungus for long timepreservation and study the various properties of bacteria and fungus.

EXPERIMENT NO. : 4

AIM- STAINING METHODS- SIMPLE, GRAM STAINING AND ACID FAST STAINING (DEMONSTRATION WITH PRACTICAL).

THEORY-

A. Gram Stain-

The Gram stain is a degree of difference stain generally used in the microbiology laboratories that differentiate bacteria on the basis of their cell wall structure. Most bacteria can be divided into two groups base on the composition of their cell wall:

1) Gram-positive cell walls have a thick peptidoglycan layer away from the plasma membrane.

Characteristic polymers called teichoic and lipoteichoic acids stick out above the peptidoglycan

And it is for the reason that of their negative charge that the cell wall is in general negative. These acids are also very essential in the body's ability to be familiar with foreign bacteria. Gram-positive cell walls

Stain blue/purple with the Gram stain.

2) Gram-negative cell walls are more multifarious. They have a thin peptidoglycan layer and an outer membrane beyond the plasma membrane. The gap between the plasma membrane and the outer membrane is called the periplasmic space. The outer leaflet of the outer membrane is composed largely of a molecule called lipopolysaccharide (LPS). LPS is an endotoxin that is vital in triggers the body's immune response and contributing to the on the whole negative charge of the cell.

on both sides of the outer membrane are porin proteins that enable the passage of small molecules.

Lipoproteins connect the outer membrane and the thin peptidoglycan layer. Gram-negative cellswill

Stain pink with the gram stain.

PROCEDURE:

1. Using a sterile inoculating loop, add 1 drop of sterile water to the slide. Prepare a mixed smear of *Escherichia coli* (G- rod) and *Staphylococcus epidermidis* (G+ coccus).
2. Air dry and Heat fix.
3. Cover up the smear with Crystal Violet (primary stain) for 1 min.
4. Softly wash off the slide with water.
5. Gram's Iodine (mordant) adds for 1 min.
6. Wash with water.
7. Decolorize with 95% ethanol. This is the "complicated" step. Discontinue decolorizing with alcohol as rapidly as the purple color has stopped leaching off the slide (time will vary dependingon thickness of smear). Without delay wash with water. Be sure to dispose of all ethanol waste inthe suitably labeled waste container.
8. Cover up up the smear with Safranin for 30 seconds.
9. Wash down both the top & the bottom of the slide with water.
10. Blot the slide with bibulous paper.
11. By means of the 10X objective lens, focus first on the line and then on the smear.

B. Acid-fast Stain-

THEORY-

Mycobacterium and many *Nocardia* species are call acid-fast because during an acid-fast staining procedure they retain the primary dye carbol fuchsin despite decolorization with the influential solvent acid-alcohol (95% ethanol with 3% HCl). Almost all other genera of bacteria are nonacid-fast. The acid fast genera have the waxy hydroxy-lipid called mycolic acid in their cell walls. It is unspecified that mycolic acids prevent acid-alcohol from decolorizing protoplasm.

The acid-fast stain is a differential

1. Add one loopful of sterile water to a microscope slide.
2. Create a heavy smear of *Mycobacterium smegmatis*. Mix thoroughly with your loop. Then remove a small amount of *Staphylococcus epidermidis* to the similar drop of water.
   You will now have a mixture of *M. smegmatis* and *S. epidermidis.*
3. Air dry and heat fix well.
4. Cover up the smear with carbolfuchsin dye. put a piece of paper towel on top of the dye. Be definite the paper towel is saturated with the dye. Carbolfuchsin is a potential carcinogen. Please have on gloves when working with this dye.
5. Put the slide on the rack over dry heat for 2 minutes.
6. Cool and rinse with water.
7. Decolorize by insertion a drop of acid alcohol on the slide and allowing it to sit for 15seconds.
8. Wash down the top and bottom of slide with water and clean the slide bottom well.
9. Counterstained with Methylene Blue for 30 seconds to 1 minute.
10. Wash down and blot the slide with bibulous paper.
11. Focus 10X - then utilize oil immersion.

RESULT-

DIFFERENTIAL STAINS

A. Gram Stain

Draw and label examples of *Escherichia coli* and *Staphylococcus epidermidis.*

B. Acid-fast Stain

Draw and label examples of *Mycobacterium smegmatis* and *Staphylococcus epidermidis*.

Mycobacterium smegmatis & *Staphylococcus Epidermidis.*

Experiment No. : 5

AIM - ISOLATION OF PURE CULTURE OF MICROORGANISMS BY MULTIPLE STREAKPLATE TECHNIQUE AND OTHER TECHNIQUES**.**

REQUIREMENTS-

A cause of bacteria, Inoculation loop, A striker/lighter, Lysol (10%v/v), Bunsen burner,Agar plate (nutrient agar or any other agar medium), Paper towels.

INTRODUCTION-

A pure culture theoretically contains a single bacterial species. There are a numeral of procedures available used for the isolation of pure cultures from mixed populations. A pure culture may be isolated by the use of special media with specific chemical or physical agents that allow the enrichment or selection of one organism over another. The most commonly used methods are-

1. Streak-plate method
2. Pour-plate method
3. Spread-plate method

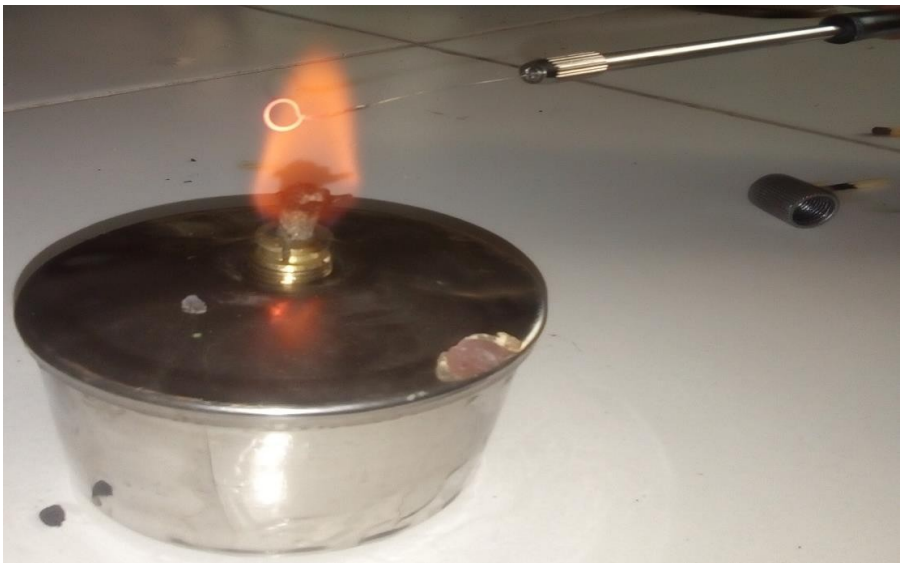
1) **Streak- plate method-**

Principle-

The sample is diluted by streaking it across the surface of the agar plate. While streaking in successive areas of the plate, the inoculums is diluted to the point where there is only one bacterial cell deposit every few millimeters on the surface of the agar plate. When these lone bacterial cells divide and give rise to thousands and thousands of new bacterial cells, an isolated colony is produced. Pure cultures can be obtained by picking well-isolated colonies and re- streaking these on fresh agar plates.

Procedure-

- Sterilize the inoculating loop in the burner by clicking on the loop and dragging it to the burner. Situate the loop into the flame until it is red hot. Allow it to cool.
- Choose an isolated colony from the agar plate culture and spread it over the first quadrant (approximately 1/4 of the plate) using close parallel streaks.
- Flame the loop.
- Turn the plate 90° and lightly sweep the loop 1-2 times through the inoculated area, then streak into the next quadrant without overlapping the previous streaks. 5.
- Flame the loop.
- Turn the plate 90°, overlap the previous area 1-2 times, and streak into the next quadrant as in step 4.
- Flame the loop.
- Streaking the residue of the plate.
- Invert the plate and incubate at 37°C for 24 hr.



2. Pour plate method

Requirements-

- 24 hours old nutrient broth culture of two or more bacteria (Mixed Culture) or Sample/Specimen.
- Nutrient Agar Medium
- Six 9 ml Sterile Water Blanks
- Sterile Petri plates
- Marker
- Graduated pipette (1m)

PROCEDURE-

Initially melt the nutrient agar medium and maintain it in the water bath at the temperature of 45
°C. Label the 6 Sterile Water blanks (9ml sterile water in each tube) as number 1 to 6 with the help of Marker. Also, label the Sterile Petri plates as number 1 to 6. Place the labeled tubes in thetest tube rack. Mix well the 24 hours old broth culture to equally distribute the bacterial cells in the tube.

After mixing, Remove the Cotton plug and aseptically transfer the 1 ml of the bacterial suspension from the tube of culture to sterile water blank tube no.1 using a graduated pipette. Shake the tube no.1 to mix well the content to uniformly distribute the bacterial cells. Transfer the 1 ml of this to the water blank tube no.2 by using the graduated pipette. In this way, make serial dilutions till the six water blanks (no. 1 to no. 6).

Transport 1 ml of the bacterial suspension each from the tube no. 1 to 6 to Petri Plates labeled as 1 to 6 by using separate sterile pipette whenever. Now, take out the Molten Agar Medium (at 45°C) from the water bath and pour the medium into the Plates no. 1-6 containing the specimen at different dilutions. Rotate the plate gently to make sure the uniform distribution of cells in the

media plates. Allow the medium to solidify at temperature. Incubate the inoculated media platesfor 24-48 hours at 37 °C in an overturned position.

3. Spread-plate method

Spread plate technique is that the method of isolation and enumeration of microorganisms in a mixed culture and distributing it evenly. The technique makes it easier to quantify bacteria during a solution.

PROCEDURE-

1. Get three agar plates and label them with the name of organisms to be inoculated.
2. Create a dilution series from a sample.
3. Pipette out 0.1 ml from the fitting desired dilution series into the middle of the surface of an agar plate.
4. Dip the L-shaped glass spreader into alcohol.
5. Flame the glass spreader (hockey stick) over a Bunsen.
6. Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petridis underneath at the equivalent time.
7. Incubate the plate at 37°C for twenty four hours.
8. Analyze the CFU value of the sample. Once you count the colonies, multiply by the suitable dilution factor to work out the amount of CFU/mL within the original sample.

Result- Some colonies are going to be seen that grow individually without over lapping with other colonies.

Experiment No. : 6

AIM- MICROBIOLOGICAL ASSAY OF ANTIBIOTICS BY CUP PLATE METHOD OR OTHERMETHODS.

The microbiological assay is based upon an evaluation of the inhibition of growth of microorganisms by measured concentrations of the standard preparation of the antibiotics to be examine with the formed by known concentrations of a regular preparation of the antibiotic having a acknowledged movement.

REQUIREMENTS– Petri plates, Borer, DMF solvent, antibiotics, antibiotic disc, nutrient agar medium

PROCEDURE-

Cup plate method

- Prepare nutrient agar plate inoculated with test organism, with a depth of 4-5 mm and then permitted it to solidify. Separate the NA plate into four equal portions.
- Then with the help of a sterile borer make four cavities one in each portion.
- Then fill three cavities with antibiotic solution and in one fill the standard solution.
- Then slowly incubate the plates at 37 c for 24 hours.
- After incubation measure the zone of inhibition.

Disk diffusion method-

- Prepare nutrient agar plates and with the help of a cotton swab spread the broth solution containing the test microorganism uniformly, then with the help of a sterile forceps put the essential antibiotic disc slowly above the plate.
- Then incubate the plate at 37 c for 24 hours.
- Calculate the one of inhibition formed.

Experiment No. : 7

AIM - MOTILITY DETERMINATION BY HANGING DROP METHOD.

REQUIREMENTS- Cavity slide, petroleum jelly or Vaseline, cover slip, immersion oil, 24-hour old broth culture of bacteria, loop and microscope.

PROCEDURE-

1. A cavity slide is clean correctly under tap water, such that water does not stay behind as drops on its surface. A cavity slide is a glass slide with a small round misery at the center, into which a little drop of bacteria suspension can fall.
2. The slide is dried out by wiping with bibulous paper and next, moving it over flame or maintenance it in the sun.
3. A ring of Vaseline is applied around the cavity.
4. A loop is sterilized above flame and let it to be cooled. A little drop of the suspension is placedat the center of a cover slip. The broth culture be supposed to not be more than 24 hours old, for the reason that bacteria may lose their motility, as they grow older.
5. The cavity slide is placed on the cover slip in such a way that, the cavity covers drop.
6. The slide and cover slip pressed together gently, so that the cavity is sealed. Carefully, shouldbe taken to see that no part of the cavity touched the drop.
7. The slide change to the opposite position such that, the drop hangs into the cavity withouttouching it.
8. The slide is abrupt to stage of the microscope.
9. The edge of the drop is focused under low pressure objective.

OBSERVATIONS -

1- Motility: motile or non motile.

2- Shape of bacteria: spherical (coccus), Rod – shaped (bacilli), comma – like (vibrio) spiral(spirochetes).

3- Arrangements of bacteria : Pairs (diplobacillus/diplococcus) In fours (tetrads) In chains (streptococcus/streptobacillus)Grape-like clusters (staphylococcus).

Size of bacteria: By eye judgment, make picture of the field under oil-immersion object.

Experiment No. : 8

AIM- TO DETERMINE THE STERILITY TEST FOR PHARMACEUTICALS

REFERENCE- As per British Pharmacopoeia or USP/ BP – Appendix XVI A. Sterility/

THEORY: The sterility of manufactured goods is defined as the nonattendance of viable and actively multiplies microorganisms when experienced in specific culture media. The test is practical to substance, preparations or articles which, according to the Pharmacopoeia, are necessary to be sterile.

PROCEDURE-

1. Fluid Thioglycollate medium (FTM)
2. Soybean Casein Digest Medium

METHOD-

DIRECT INOCULATION

- The test article is directly inoculated into two types of media to allow for the detection of both aerobic and anaerobic microorganisms.
- After inoculation, both media types are incubated for 14 days. alternating observations as well as a ultimate examination at the end of the testing phase are conduct to detect evidence of microbial contamination.

MEMBRANE FILTRATION

- Sterile, enclosed units allow for the simultaneous filtration of equal volumes of test samples through two membrane filters.
- Samples are incubated in two types of media for 14 days, facilitating the detection of both aerobic and anaerobic microorganisms.

In the sterility examination, two dissimilar media are used: fluid thioglycollate and soya-bean casein digest medium. The test is inoculated into the growth media and the test is incubate for 14 days. Bio Outsource is able to details the results of the assay with a Certificate of Analysis within5 days of the final explanation.

It be supposed to be noted that these sterility tests are not appropriate for test articles that are not proposed to be sterile; a Bioburden test should be used in this case.

Result- Sterility test for pharmaceutical product is from membrane filtration and inoculation.

Experiment No. : 9

AIM: TO DETERMINE THE BACTERIOLOGICAL ANALYSIS OF WATER.

THEORY: the water examination of water is due to utilities and many governess agencies to supply safe or pure drinking, bathing, swimming and other domestic and industrial uses.

Human fecal pathogens vary in kind (virus, bacteria, and protozoa) and many in numbers, it is very impossible to test each water sample for each pathogen. Instead it is much easier to check the presence of non pathogenic organisms such as E.coli. There vary various test for the presence of absence of coliforms. In coliform there is comprised of gram negative, nonspore-foring aerobic to facultative anaerobic rode, which form lactose o acid and gas.

STANDARD WATER ANALYSIS

THE PRESUMPTIVE TEST

In the succession of this test lactose broth tubes are inoculated through the water sample to be tested. In this series of tube contain at least 3 to 5 tube .More tubes are sensitive to the test. If there is presence of gas then it is presumptive evidence for the presence of coliforms.

THE CONFIRMED TEST

The tubes inoculated with the water sample produced gas the water are to be unsafe. It is possible that the formation of gas may due to presence of coliform. Presence of coliforms necessary to inoculate EMB (erosin methylene blue) agar plate form positive presumptive test. This EBM inhibit gram positive organism and allowed gram negative coliform to grow.

THE COMPLETED TEST

In this test organism grow n confirmed test medium. The organism are used a nutrient agar a tube of lactose broth. After 24 hours at 37 Celsius lactose check for production of gas. Organism is gram negative nonspore form rod produced gas in lactose tube then positive coliform are here in water sample.

First periodMaterial

1. Nine tube of double strength lactose broth.
2. 10, 1.0 and 0.1ml pipettes.
3. Water sample.

PROCEDURE

PRESUMPTIVE TEST

1. First we take water sample and 3 inoculated tubes of lactose broth with 10 ml and 3 of 1.0 ml and 3 of 0.1 ml of tubes.
2. Incubate all tube t 37 Celsius for 24 hours.

SECOND PERIODMATERIAL

1. EMB agar plate PROCEDURE PRESUMPTIVE TEST

1. Observe all tubes at each dilution show hat gas production in 24 hours.
2. Set for an additional for 24 hours at 37 Celsius.

CONFIRMED TEST

1. First we inoculate an EMB plate with material from a tube containing gas.
2. Fill an incubate the plate at 37 Celsius for 24 hours.

THIRD PERIODMATERIAL

1. LACTOSE BROTH TUBE
2. NUTRIENT AGAR SLANTSPROCEDURE PRESUMPTIVE TEST

1. Observe all the tubes at each dilution that show gas record the result and determine the mostprobable number index.

CONFIRMED TEST

1. Observe the EMB agar plate a positive established test indicated by small colonies with dark center and green metallic sheen.

COMPLETED TEST

1. Firstly we take inoculate a lactose broth tube and a nutrient agar slant with organism from the EMB plate.
2. Then we incubate the broth tube and agar slant at 37 Celsius for 24 hours.

FOURTH PERIOD PROCEDURE COMPLETED TEST

1. Check for the gas production in lactose broth tube.
2. Then we create a gram stain from the organism on the nutrient agar slant.
3. Record the result

EXPERIMENT NO. : 10

AIM- BIOCHEMICAL TEST

PROCEDURE-

1. Catalase test

Enzymes with the purpose of decompose hydrogen peroxide into water and oxygen.

Hydrogen peroxide forms as one of the oxidative end yield of aerobic carbohydrate metabolism. If this is tolerable to build up in the bacterial cells it become lethal to the bacteria

Reagent 3% hydrogen peroxide store in dark brown bottle underneath refrigeration, 18 to 24 hrs. Culture of the organism to be tested.

Examples: Positive: Staphylococcus aurous; Negative: Streptococcus spp.

2. Oxidase test

To establish the presence of bacterial cytochrome enzyme oxidase.

Cytochromes in aerobic respiration transfer electrons (H) to oxygen to form water.

Dye p-phenylenediamide dihydrochloride reagent used in the presence of enzyme cytochrome oxidase dye is used. Indophenol blue which is a dark purple colored yield.

3. Indole test

Indole is a component of the amino acid tryptophan. Bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase. When tryptophan is broken down, the presence of indole can detected through the use of Kovacs' reagent. Kovac's reagent, which is yellow, reacts with indole and produces a red color on the surface of the test tube.

4. Methyl red

The steady construction of an adequate amount of acid to conquer the phosphate buffer will effect in a pH of below 4.4. If the pH indicator (methyl red) is added to an aliquot of the culture broth and the pH is below 4.4, a red color will become visible (first picture, tube on the left).

If the MR turns yellow, the pH is above 6.0 and the varied acid fermentation pathway has not been utilized (first picture, tube on the right).

The 2, 3 butanediol fermentation corridor will ferment glucose and produce a 2, 3 butanediol endcreation as an alternative of organic acids.

In order to test this alleyway, an aliquot of the MR/VP culture is unconcerned and - naphthol and KOH are added.

RESULT-

Biochemical test used in pharmaceuticals products and industry.

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