आईएफटीएम विश्वविद्यालय, मुरादाबाद, उत्तर प्रदेश

IFTM University, Moradabad, Uttar Pradesh

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E-Content

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Pharmaedu

Pharmaceutical Microiology,BP307P,Lab Manual

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Experiment No:-1

Aim:- To study the protection of yourself and clothes.

Principle:- (1) **Protection of yourself and cloths-**

- A laboratory coat or aprin must be wear at all time.
- Long hair must be secure from Bunsen burner and contamination from laboratory culture.
- Report all accident and injuries as soon as possible.
- All personal items which are not required for laboratory should be placed outside.
- Use face mask, surgical gloves whenever conducting experiments with microorganisms.

(2) **Sanitary Precaution**:-

- Before starting the experimental work scrub down the top of your table or working area with disinfectant. This will remove dust worn microorganism which may contaminate the experiment or culture.
- Repeat the scrub down procedure at the end of the experiment to remove all microorganism that might have been unknowingly split from culture.
- Do not drink or eat food in the laboratory. Make it a habit to keep your hands away from mouth.
- Observe strict sanitary procedure with respect to handling glasswares.
- Place old cultures or used glasswares on a separate place that are to be autoclaved.
- Whenever bacterial cultures are accidentally split on floor notify the laboratory incharge for the proper disinfection procedure to be assured.
- Do not remove culture and other material from the laboratory.
- Before leaving the laboratory wash your hands with soap and water

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Experiment No:-2

Aim: To study the sterilization methods and equipments.

References: 1. R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6th edition, 2000, pg. no. 23-32.

2. Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, 5th edition, pg. no. 469-509.

Theory:

Sterilization:- Sterilization is defined as the process by which an article, surface or medium is freed of all living microorganism either in the vegetative or spore state.

The various agents used in sterilization can be classified as follows-

A- Physical agents

1. Sunlight
2. Drying
3. Dry heat: flaming, incineration, hot air.
4. Moist heat: Pasturization, boiling, stem under normal pressure, steam under pressure.
5. Filteration: candles, asbestos pads, membranes.
6. Radiation
7. Ultrasonic and sonic vibrations

B- Chemicals

1. Alcohols: ethyl alcohol, isopropyl alcohol, trichlorobutanol.
2. Aldehydes: formaldehyde, glutraldehyde.
3. Dyes
4. Halogens
5. Phenols
6. Surface active agents

7. Metallic salts
8. Gases: ethylene oxide, formaldehyde, betapropiolactone.

A. Physical agents

1. Sunlight

Sunlight posses appreciable bacterial activity and plays an important role in spontaneous sterilization that occurs under natural conditions. The action is primarily due to its content of ultraviolet rays.

2. Drying

Moisture is essential for the growth of bacteria. Drying in air has therefor a deleterious effect on many bacteria. However, this method is unreliable and is only of theoretical interest. Spores are unaffected by drying.

3. Heat

Heat is the most reliable method of sterilization and should be the method of choice unless contraindicated. Materials that may be damaged by heat can be sterilized at lower temperatures for longer periods or by repeated cycles. The factors influencing sterilization by heat are-

1. Nature of heat: dry heat or moist heat.
2. Temperature and time
3. Number of microorganisms present
4. Characteristics of organisms such as species, strain, sporing capacity
5. Type of material from which the organism have to be eradicated.

The time required for sterilization is inversely proportional of the temperature of exposure and can be expressed as “thermal death time”, which is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

4. Dry Heat

Flaming- Inoculating loop or wire, the tip of forceps and serving spatula’s are held in a Bunsen burner till they become red hot. Inoculating loops carrying infective material may be dipped in a disinfectant before flaming spattering.

Incineration- This is an excellent method for safely destroying materials such as contaminated cloth, animal carcasses, and pathological materials. Plastic such as PVC and polyethylene can dealt with similarly but polystyrene materials emit clouds of dense black smoke and hence should be autoclaved in appropriate containers.

Hot air oven- This is the most widely used method of sterilization by the dry heat. A holding period of 160^0c for one hour is used to sterilize glass ware, forceps, scissors, scalpels, all glass syringes, swabs, some pharmaceutical productssuch as liquid paraffin dusting powder, fats and greese. Hot air is a bad conductor of heat and its penetrating power is low. The oven is usually

heated by electrically with heating elements in the wall of the chamber. It must be fitted with a fan to ensure distribution of air and elimination of air pockets(figure 1.1). It should be arranged so as to allow free circulation of air in between the objects. Glassware should be perfectly dry before being placed in the oven. Test tubes and flasks should be wrapped in paper. Rubber material except silicon rubber, will not withstand the temperature. At 180^0C cotton plug may get charred. The British Pharmacopoeia recommends a holding time of one hour at 150^0C for oils, glycerol and dusting powder. The oven must be allowed to cool slowly for about two hours before the door is opened since the glass ware may get crack due to sudden or uneven coolings.

Sterlization control:- The spores of a nontoxigenic strain of *Clostridium tetani* are used as a microbiological test of dry heat efficiency. Paper strips impregnated with 10^6 spores are placed in envelopes and inserted into suitable packs. After sterilization, the strips are removed and inoculated into thioglycollate or cooked meat media and incubated for sterility test under strict anaerobic conditions for five days at 37^0C.

A browne's tube(green spot) is available for dry heat and is convenient for routine use. After proper sterilization a green colour is produced(after 60 minutes at 160^0C or 115 minutes at 150^0C).

5. Moist Heat

Temperature below 100^0C- For *pasteurization* of milk: The temperature employed is either 63^0C for 30 minutes(the holder method) or 72^0C for 15-20 seconds(the flash process) followed by cooling quickly to 13^0C 0r lower. By these process all nonsporing pathogens such as mycobacteria, brucellae and salmonellae are destroted. *Coxiella burcnetti* is relatively heat resistant and may survive the holder method.

Media such as Lowenstein-Jensen and Loffler's serum are rendered sterile by heating 80-85^0C for half an hour on three successive days in an inspissator.

Vaccines of nonsporing bacteria are heat inactivated in special vaccine baths at 60^0C for one hour. Serums or body fluids containing coagulable proteins can be sterilized by heating for one hour at 56^0C in a water bath on several successive days.

Though practically all mesophilic nonsporing bacteria are killed by exposure to moist heat at 60^0C for 30 minutes, *Staphylococcus aureus* and *Streptococcus faecalis* require 60 minutes.A temperature of 80^0C for 5-10 minutes destroys the vegetative forms of all bacteria, yeasts and moulds. Heat resistant cells of *Clostridium botulinum* which require 120^0C for four minutes, or 100^0C for 330 minutes for their destruction.

Temperature at 100^0C- Boiling: Vegetative bacteria are killed almost immediately at 90-100^0C, but sporing bacteria require prolonged periods of boiling. Boiling is not recommended for the sterilization of instruments used for surgical procedures and should be regarded only as a means of disinfection. Sterilization may be promoted by the addition of 2% Sodium bicarbonate to the water.

Steam at atmospheric pressure(100^0C)- An atmosphere of free steam is used to sterilize culture media which may decompose if subjected to higher temperatures. A Koch or Arnold steamer consists of a tinned copper cabinet with the walls suitably lagged. The lid is conical, enabling drainage of condensed steam, and a perforated tray fited above the water level ensures that the material placed on it is surrounded by steam. A single exposure of ninety minutes usually ensures sterilization but for media containing sugars or gelatin an exposure of 100^0C for 20 minutes on three successive days is used. This is known as tyndallisation or intermittent sterilization. The principle is that the first exposure kills all vegetative bacteria, and the spores, since they are in a

favourable medium, will germinate and be killed on the subsequent occasions. This method may fail with spores of certain anaerobic and thermophiles.

Steam under pressure- The principle of the autoclave or steam sterilization is that water boils when its vapour pressure equals that's of the surrounding atmosphere. Hence when pressure inside a closed vessel increase, the temperature at which water boils also increases. Saturated steam has penetrating power. When steam comes into contact with a cooler surface it condensed to water and gives up its latent heat to that surface. The condensed water ensures moist conditions for killing the microbes present.

Sterilization by steam under pressure is carried at temperatures between 108^0C and 147^0C. by using the appropriate temperatures and time, a variety of materials such as dressings, instruments, laboratory ware, media and pharmaceutical products can be sterilized. Aqueous solutions are sterilized between 108^0C and 126^0C. heat is conducted through the walls of the sealed containers until the temperature of the fluid inside is the same as that of steam outside.

Several types of steam sterilizers are in use:-

1. Laboratory autoclaves,
2. Hospitals dressing sterilizers,
3. Bowl and instrument sterilizer, and
4. Rapid cooling sterilizers.

In its simplest form, the laboratory autoclave consists of a vertical or horizontal cylinder of gunmetal or stainless steel, in a supporting sheet iron case. The lid or door is fastened by screw clamps and made airtight by an asbestos washer. The autoclave has on its lid or upper side a discharge tap of air and steam, a pressure gauge and a safety valve that can be set to blow off at any desired pressure. Heating is by electricity.

Sufficient water is put in the cylinder, the material to be sterilized is placed on the tray, and the autoclave is heated. The lid is screwed tight with the discharge tap open. The safety valve is adjusted to the require pressure. The steam-air mixture is allowed to escape freely till all the air has been displaced. When no more air bubbles come out in the pail the discharge tap is closed. The steam pressure rises inside and when it reaches the desired set level, the safety valve opens and the excess steam escapes. From this point, the holding period is calculated. When the holding pressure is over, the heater is turned off and the autoclave allowed to cool till the pressure gauge indicates that the pressure inside is equal to the atmospheric pressure. The discharge tap is opened slowly and air is let into the autoclave. If the tap is opened when the pressure inside has fallen below atmospheric pressure, an excessive amount of water would have evaporated and lost from the media.

Sterilization control: For determining the efficacy of moist heat sterilization, spores of *Bacillus stearothermophilus* are used as the test organism. This is a thermophilic organism with an optimum growth temperature of 55-60^0C and its spores require an exposure of 12 minutes at 121^0C to be killed. Paper strips impregnated with 10^6 spores are dried at room temperature and placed in paper envelopes. These envelopes are inserted in different parts of the load. After sterilization, the strips are inoculated into a suitable recovering medium and incubated for sterility test at 55^0C for five days.

6. Filteration

Filteration helps to remove bacteria from heat labile liquids such as sera and solutions of sugars or antibiotics used for preparation of culture media. As viruses pass through ordinary filters,

filteration can be used to obtain bacteria-free filtrates of clinical samples for virus isolation. The following types of filters have been used:-

1. Candle filters
2. Asbestos filters
3. Sintered glass filters
4. Membrane filters

7. Radiation

Two types of radiation are used for sterilization, non-ionising and ionizing. Infrared and ultraviolet rays are of the nonionising low energy type, while gamma rays and high energy electrons are the high energy ionizing type.

8. Ultrasonic and sonic vibrations

Ultrasonic and sonic vibration are credited with bactericidal powers but the result have been variable. Microorganisms vary in their sensitivity to them and survivors have been found after such treatment. Hence this method is of no practical value in sterilization and disinfection.

B. Chemical agents

Several chemical agents are used as antiseptics and disinfectants. However little is known about the mechanism of action of many of these agents.

1. Alcohols

Ethyl alcohol and isopropyl alcohol are the most frequently used. They are used mainly as skin antiseptics and act by denaturing bacterial proteins. They have no action on spores. To be effective they must be used at a concentration of 60-70% in water. Isopropyl alcohol is preferred as it is a better fat solvent, more bactericidal and less volatile. It is used for the disinfection of clinical thermometers.

2. Aldehydes

Formaldehyde is active against the amino group in the protein molecule. In aqueous solution, it is markedly bactericidal and sporicidal and also has a lethal effect on viruses. Glutraldehyde has an action similar to formaldehyde. It is specially effective against tuber bacilli, fungi and viruses. It is less toxic and irritant to the eyes and skin than formaldehyde.

3. Dyes

Two groups of dyes, aniline dyes and acridine dyes are used extensively as skin and wound antiseptics. Both are bacterostatic in high dilution but are of low bactericidal activity. The aniline dyes in use are brilliant green, malachite green and crystal violet. They are more active against gram positive organisms than gram negative organisms. The acridine dyes are more active against gram positive organisms than gram negative but are not as selective as the aniline dyes.

4. Halogens

Iodine in aqueous and alcoholic solution has been widely used as a skin disinfectant. It is actively bactericidal with moderate action against spores. It is active against the tubercle bacteria and viruses. Compounds of iodine with nonionic wetting or surface active agents known as

iodophores are claimed to be more active than the aqueous or alcoholic solutions of iodine. Chlorine and its compound have been used as disinfectants for many years.

5. Phenols

They are obtained by distillation of coaltar between temperature 0f 170^0C and 270^0C. the lethal effect of phenol is due to their capacity to cause cell membrane damage, releasing cell contents and caused lysis. Low concentration of phenol precipitate proteins. Membrane bound oxidases and dehydrogenases are inactivated by concentrations os phenols that are rapidly bactericidal for microbes.

6. Gases

Ethylene oxide- This is a colorless liquid with a boiling point 10.7^0C, and at normal temperature and pressure is a highly penetrating gas with a sweet ethereal smell. Its action is due to its alkylating the amino, carboxyl, hydroxyl, and sulphydryl groups in protein molecules.

Formaldehyde gas- This is widely used for fumigation of operation theatres and other rooms.

7. Surface active agents

Substances which alter energy relationship at interfaces, producing a reduction of surface or interfacial tension are reffered to as surface active agents.the most important antibacterial agents are the cationic surface active agents. These act on the phosphate groups of the cell membrane and also enter the cell. The membrane loses its semipermeability and the cell proteins are denatured. The cationic compounds in the form of quaternary ammonium compounds are markedly bactericidal, being active against gram positive organisms and to a lesser extent on gram negative ones. Soaps prepared from saturated fatty acids(coconut oil) are more effective against gram negative bacilli while those prepared from unsaturated fatty acids(oleic acid) have greater action against gram positive and Neisseria group of organisms.

8. Metallic salts

Though all salts have a certain aount of germicidal action depending on their concentration, salts of heavy metals have a greater action. The salts of silver, copper, and mercury are used as disinfectants.

Result:- All the sterilization methods and equipments were studied.

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Experiment No:-3

Aim: To study the preparation and sterilization the following media

a. Nutrient broth media

b. Nutrient agar medium

c. Yeast extract media

d. General purpose medium

References:- 1. R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6th edition, 2000, pg. no. 33-37.

2. Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, third reprint 2011, pg. no. 142

Requirements:- Conical flask, glass rod, beaker, autoclave and chemical, volumetric flask.

Theory:

A microbiological medium is the food that is used for culturing bacteria, molds and other microorganisms. In order to grow, all organisms need a variety of chemical elements as nutrients. These elements are necessary for both the sysnthesis and the normal functions of cellular components. They exist in nature in a great variety of compounds, which are either inorganic or organic. When microorganisms are removed from their environment and cultivated in the laboratory, microbiologists use media that stimulate or even improve on natural conditions. The main elements for cell growth include carbon, nitrogen, hydrogen, oxygen, sulfur, and phosphorus.

Three physical forms media are used: liquid, or broth media; semisolid media; and solid media. The major difference among these media is that solid and semisolid media contain a solidifying agent (usually agar), whereas a liquid medium does not.

1. Liquid media- The media which contain liquefiable agent and become or behave as liquid at room temperature. Liquid media, such as nutrient broth, tryptic soy broth, or brain-heart infusion broth can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests.
2. Solid media- These are made by adding a solidifying agents such as agar, gelatin or silica gel to a liquid medium(generally distilled water) and get solidify at room temperature and require high temperature for melting. Nutrient agar , blood agar, sabourauds agar are the examples of solid media that are used for developing surface colony growth of bacteria and molds.
3. Semisolid media- It falls in between liquid and solid media. Although they are similar to solid media in that they contain solidifying agents such as agar and gelatin but semisolid media are more jelly like due to lower percentage of these solidifiers. e.g. 0.2-0.5% agar concentration in semisolid.

Types of media

Media can be classified into three categories on the basis of chemical components. These are:-

1. Synthetic media 2. Complex media 3. Natural media

1. Synthetic media or defined media: These media are prepared from pure chemical substances and the exact composition of the medium is known. These are used for various

special studies such as metabolic requirements. Simple peptone water medium, 1% peptone with 0.5% NaCl in water, may be considered a semidefined medium since its composition is approximately known.

2. Complex media: These have added ingredients for special purposes or for bringing out certain characteristics or providing special nutrients required for the growth of the bacterium under study.
3. Natural media: Substrates of natural origin that favors microbial growth are employed in this media. e.g. milk.

Media can be classified based on their functional properties. They are as follows:-

1.Simple media 2. Differential media 3. Selective media 4. Enriched media 5. Enrichment media 6. Indicator media 7. Sugar media 8. Transport media 9. Anaerobic media.

1. Simple media(basal media): an example is nutrient broth. It consists of peptone, meat extract, sodium chloride and water. Nutrient agar, made by adding 2% agar to nutrient broth is the simplest and most common medium in routine diagnostic laboratories.

2. Differential media: A medium which has substances incorporated in it, enabling it to bring out different characteristics of bacteria and thus helping to distinguish between them, is called a differential medium. for example MacConkey's medium which consists of peptone, lactose, agar, neutral red and taurocholate shows up lactose fermenters as pink colonies, while nonlactose fermenters are colourless or pale. This may also be termed as indicator medium.

3. Selective media: These type of media selectively allow the growth of particular type of organism and prevent the growth of most of other microbes. for example desoxycholate citrate medium for dysentery bacilli.

4. Enriched media: In these media, substances such as blood, serum, or egg are added to a basal medium. They are used to grow bacteria which are most exacting in their nutritional needs. Examples are blood agar, chocolate agar, and egg agar.

5. Enrichment media: In mixed cultures or in materials containing more than one bacterium, the bacterium to be isolated is often overgrown by the unwanted bacteria. For example *S.typhi* being overgrown by *E. coli* in cultures from feces. In such situations, substances which have a stimulating effect on the bacteria to be grown or an inhibitory effect on those to be suppressed are added to a liquid medium, the result is an absolute increase in the numbers of the wanted bacterium relative to the other bacteria. Such media are called enrichment media, for example tetrathionate broth.

6. Indicator media: These media contain an indicator which changes colour when a bacterium grows them, for example incorporation of sulphite in Wilson and Blair medium. *S. typhi* reduces sulphite to sulphide in the presence of glucose and the colonies of *S. typhi* have a black metallic sheen.

7. Sugar media: The term 'sugar' in microbiology denotes any fermentable substance. The usual sugar media consists of 1% of the sugar in peptone water along with an appropriate indicator.

8. Transport media: In case of delicate organisms(like gonococci) which may not survive the time taken or transporting the specimen to the laboratory or may be overgrown by nonpathogens, for these type of microorganisms special media are devised for transporting the specimens. For example Stuart's medium.

9. Anaerobic media: These media are used to grow anaerobic organisms. For example Robertson's cooked meat medium.

Method of preparation

a. For Nutrient broth media

Formula

Peptone	10 gm
Sodium chloride	5 mg
Beef extract	10 gm
Distilled Water up to	1000ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. pH is adjusted to 8.0 to 8.4 and the solution was boiled for 10min. the prepared medium was then filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 7.3±0.1.

b. Nutrient agar medium: Nutrient broth gelled by the addition of 1-2% w/v of agar.

Formula

Peptone	10 gm
Sodium chloride	5 mg
Beef extract	10 gm
Agar	12 gm
Distilled Water up to	1000ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. pH is adjusted to 8.0 to 8.4 and the solution was boiled for 10min. The prepared medium was then filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 7.4±0.1.

c. Yeast extract media

Formula

Malt extract	0.3 gm
Glucose	1.0 gm
Yeast extract	0.3 gm
Peptone	0.5 gm
Agar	2.0 gm
Distilled water upto	100 ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. The prepared solution was then boiled for 10min. The prepared medium was then filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 6.4-6.8.

d. General purpose medium, sabouraud's agar: for the isolation and growth of fungi

Formula

Peptone 10gm

Glucose 20gm

Agar 25gm

Distilled Water upto 1000ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. The prepared solution was then boiled for 10min. The prepared medium was filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 5.6.

Result:- Various culture media was prepared and sterilized for the cultivation of aerobic and anaerobic bacteria for different experiments.

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Experiment No:-4

Aim: To prepare and sterilize Nutrient broth media

References:- 1. R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6^{th} edition, 2000, pg. no. 33-37.

2. Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, third reprint 2011, pg. no. 142

Requirements:- Conical flask, glass rod, beaker, autoclave and chemical, volumetric flask.

Theory:

A microbiological medium is the food that is used for culturing bacteria, molds and other microorganisms. In order to grow, all organisms need a variety of chemical elements as nutrients. These elements are necessary for both the sysnthesis and the normal functions of cellular components. They exist in nature in a great variety of compounds, which are either inorganic or organic. When microorganisms are removed from their environment and cultivated in the laboratory, microbiologists use media that stimulate or even improve on natural conditions. The main elements for cell growth include carbon, nitrogen, hydrogen, oxygen, sulfur, and phosphorus.

Three physical forms media are used: liquid, or broth media; semisolid media; and solid media. The major difference among these media is that solid and semisolid media contain a solidifying agent (usually agar), whereas a liquid medium does not.

4. Liquid media- The media which contain liquefiable agent and become or behave as liquid at room temperature. Liquid media, such as nutrient broth, tryptic soy broth, or brain-heart

infusion broth can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests.

5. Solid media- These are made by adding a solidifying agents such as agar, gelatin or silica gel to a liquid medium(generally distilled water) and get solidify at room temperature and require high temperature for melting. Nutrient agar , blood agar, sabourauds agar are the examples of solid media that are used for developing surface colony growth of bacteria and molds.
6. Semisolid media- It falls in between liquid and solid media. Although they are similar to solid media in that they contain solidifying agents such as agar and gelatin but semisolid media are more jelly like due to lower percentage of these solidifiers. e.g. 0.2-0.5% agar concentration in semisolid.

Types of media

Media can be classified into three categories on the basis of chemical components. These are:-

2. Synthetic media 2. Complex media 3. Natural media

4. Synthetic media or defined media: These media are prepared from pure chemical substances and the exact composition of the medium is known. These are used for various special studies such as metabolic requirements. Simple peptone water medium, 1% peptone with 0.5% NaCl in water, may be considered a semidefined medium since its composition is approximately known.
5. Complex media: These have added ingredients for special purposes or for bringing out certain characteristics or providing special nutrients required for the growth of the bacterium under study.
6. Natural media: Substrates of natural origin that favors microbial growth are employed in this media. e.g. milk.

Procedure

For Nutrient broth media

Formula

Peptone	10 gm
Sodium chloride	5 mg
Beef extract	10 gm
Distilled Water up to	1000ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. pH is adjusted to 8.0 to 8.4 and the solution was boiled for 10min. the prepared medium was then filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 7.3±0.1.

Result: Nutrient broth culture media was prepared and sterilized for the cultivation of aerobic and anaerobic bacteria for different experiments.

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Experiment No:-5

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Aim: To isolate and enumerate microorganisms by spread plate method.

References:- 1. R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6th edition, 2000, pg. no. 9.

2. Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, third reprint 2011, pg. no. 76.

Requirements:- Microscopic slide, methylene blue, crystal violet, carbol fuchsin, Inoculating loops, Broth cultures of various bacteria, Microscope.

Theory

A microbiological medium is the food that is used for culturing bacteria, molds and other microorganisms. In order to grow, all organisms need a variety of chemical elements as nutrients. These elements are necessary for both the sysnthesis and the normal functions of cellular components. They exist in nature in a great variety of compounds, which are either inorganic or organic. When microorganisms are removed from their environment and cultivated in the laboratory, microbiologists use media that stimulate or even improve on natural conditions. The main elements for cell growth include carbon, nitrogen, hydrogen, oxygen, sulfur, and phosphorus.

Procedure

For Nutrient broth media

Formula

Peptone 10 gm

Sodium chloride 5 mg

Beef extract 10 gm

Distilled Water up to 1000ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. pH is adjusted to 8.0 to 8.4 and the solution was boiled for 10min. the prepared medium was then filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 7.3±0.1.

1. Then make a dilution series from a sample.
2. Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
3. Dip the inoculating loop into alcohol.
4. Flame the inoculating loop over a Bunsen burner.
5. Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at the same time.
6. Incubate the plate at 37°C for 24 hours.
7. Observe the sample for growth of microbial colonies.

Result: The petriplates shows growth of microbial colonies they were counted.

Experiment No:-6

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Aim: To isolate and enumerate microorganisms by streak plate method.

References:- 1. R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6th edition, 2000, pg. no. 9.

2. Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, third reprint 2011, pg. no. 76.

Requirements:- Microscopic slide, methylene blue, crystal violet, carbol fuchsin, Inoculating loops, Broth cultures of various bacteria, Microscope.

Theory

A microbiological medium is the food that is used for culturing bacteria, molds and other microorganisms. In order to grow, all organisms need a variety of chemical elements as nutrients. These elements are necessary for both the sysnthesis and the normal functions of cellular components. They exist in nature in a great variety of compounds, which are either inorganic or organic. When microorganisms are removed from their environment and cultivated in the laboratory, microbiologists use media that stimulate or even improve on natural conditions. The main elements for cell growth include carbon, nitrogen, hydrogen, oxygen, sulfur, and phosphorus.

Procedure

For Nutrient broth media

Formula

Peptone	10 gm
Sodium chloride	5 mg
Beef extract	10 gm
Distilled Water up to	1000ml

All the ingredients were accurately weighed and dissolved in water with the aid of heat. pH is adjusted to 8.0 to 8.4 and the solution was boiled for 10min. the prepared medium was then filtered and sterilized by autoclaving at 115^0C for 30 minutes and then pH was finally adjusted to 7.3±0.1.

Then make a dilution series from a sample.
-Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
-Dip the inoculating loop into alcohol.
-Flame the inoculating loop over a Bunsen burner.
-Make streaks of microbial sample over the surface of agar using the inoculating loop, carefully rotating the Petri dish underneath at the same time.
-Incubate the plate at 37°C for 24 hours.
-Observe the sample for growth of microbial colonies.

Result: The petriplates shows growth of microbial colonies they were counted.

Experiment No:-7

Aim:- To study the sub-culturing of microorganism in Liquid and Solid media.

References:- 1 R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6th edition, 2000, pg. no. 33-41.

2. Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, third reprint 2011, pg. no. 134-138.

Requirements:- inoculating loop, bacterial strain, Nutrient medium, nutrient agar medium

Theory:- In order to identify and classify micro-organisms, we must learns their characteristic. It is not easy to study the characteristics of a single micro-organisms because of its small size. Therefore we generally study the characteristics of a culture, a population of a micro-organisms. A culture that consists of a single kind of micro-organisms, regardless of the number of individuals in an environment free of other living organisms is called a "pure culture". A stain is made up of all the descendents of a pure culture. If a strains is derived from a single percent cell it is called "clone". Each strains has a specific history and designation.

Sub culturing is a process of growing the strain into a nutrient media which may be either solid or liquid and makes it reproduce and grow it into a colony.

The media generally contains beef extracts, agar, peptone, sodium chloride and water is required for population. After 24hrs. The growth of the bacterial strains in the medium is notice. From sub culturing we get the idea whether the media prepared is suitable for growth or not. When the bacteria inoculation can indicate the growth called "Positive controls" and when the bacteria inoculation cannot indicate the growth called "Negative controls".

Procedure

(i) Sub culturing in a Liquid medium [Nutrient broth] :- The bacterial strain is collected from the solid culture with the help of an inoculating loop. This loop then dipped into the Liquid medium.

(ii) Sub culturing in a Solid medium [Nutrient agar]:- In the agar slant the bacterial strain was stricked with the help of an inoculating loop after collecting the strain from the solid culture medium.

Precaution

(i) The inoculating loop should be sterilised each time before use.

(ii) The inoculation should be done under aseptical condition.

(iii) Cotton plugging should be done carefully and properly after transforming the bacteria

(iv) One should use head gear and musk to avoid contamination and infection.

Result

- In case of Liquid [nutrient broth]- presence of Turbidity --- Yes/No.
- In case of Solid [nutrient agar]- presence of Haziness --- Yes/No.

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Experiment No:-8

Aim: To isolate bacteria from soil sample(Garden soil) by serial dilution method and Spread plate method.

Reference: R. Ananthanarayan, C.K.J Paniker, Text book of microbiology, published by Orient Longman Limited , Hyderabad, 6th edition, 2000, pg. no. 33-41.

Requirements: Bent glass rod, Nutrient agar culture media, inoculating loop, Isopropyl alcohol, soil sample, testubes, petridish.

Theory

Soil teems with microscopic life. A teaspoon of fertile garden soil may contain billions of microorganisms- bacteria, fungi, algae, protozoa, and viruses. Directly or indirectly, animal wastes and the dead bodies of animals and tissues of plants eventually enter the soil. In time they all disappear, transformed into substances that enrich the soil. Microorganisms are responsible for these transformations, converting dead plant and animal matter into simple inorganic substances that nourish plants.

Actinomycetes (including actinoplanetes, nocardio-forms, and streptomycetes), other bacteria, and fila-mentous fungi (Rhizopus, Mucor, Penicillium, and Aspergillus) are all important members of the soil microbial community. Protozoa, algae, cyanobacte-ria, nematodes, insects and other invertebrates, and viruses are also important members. Each gram of rich garden soil may contain millions of these micro- and macroorganisms. Since soils vary greatly with respect to their physical features (e.g., pH, general type, temperature, and other related factors), the microorganisms present will also vary. For example, acid soils will have a higher number of fungi compared to alkaline soils, and rich garden soil will contain more actinomycetes than either the other bacteria or fungi. Not surprisingly, no single technique is available to count the microbial diversity found in average garden soil.

Culture methods:- Culture method employed depend on the purpose for mwhich they are intended, in the clinical laboratory, the indications for culture are mainly to:

1. Isolate bacteria in pure cultures
2. Demonstrate their properties
3. Determination sensitive to antibiotics
4. Estimate viable count
5. Maintain stock cultures

The methods of culture used ordinarily in the laboratory are the following:-

1. Streak culture
2. The lawn or carpet culture
3. The stroke culture
4. Stab culture
5. Pour plate culture
6. Sweep plate methods
7. Liquid culture

Spread plate method

In pour plate method as well as spread plate method, the bacterial culture is first diluted to provide only few cells per millimeters before being used to inoculate media.

In Spread plate method the mixed culture is not diluted in the culture medium as in case of pour plate method; instead it is diluted in a series of tubes containing a sterile liquid usually water or physiological saline. A sample is removed from each testube and placed onto the surface of agar plate and spread evenly over the surface by means of a sterile bent glass rod. On atleast one plate of the seires the bacteria will be in numbers sufficiently low, so as to allow the development of well separated colonies.

Procedure

1. Preparation of culture media:- Nutrient agar medium was prepared same as in experiment 3
2. Procedure for dilutions:

   i. Taken 5 test tubes, washed with detergent and autoclaved for 15 minutes at 15 pounds pressure at 121^0C temperature.

   ii. Then taken 9 ml sterile water in each testube and make serially diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}.

   Note: For 10^{-1} dilution ,add 1gm soil in 9ml water and for 10^{-2}, add 1 ml solution of 10^{-1} testube into another testube which contain 9 ml water and so on.

3. Procedure for spread plate technique:

i. The bent glass rod is placed into the beaker containing isopropyl alcohol and then passed through fire, so as to sterilize the bent glass rod.

ii. Similary, the inoculating loop was sterilized.

iii. Nutrient agar plate was taken and with the help of inoculating loop. A loop full of culture from each test tube was placed on the respective petridishes.

Note- all the testtubes and petridish were properly named according to the dilution made with the help of marker pen.

iv. Again sterilize the inoculating loop as done in step i.

v. With the help of bent glass rod the inoculum was spread uniformly on the culture medium.

vi. Again sterilize the bent glass rod as done in step i.

vii. Cover the petridish and incubate them at 37^0C for 24 hours for the development of colonies.

viii. After 24 hours, all petridishes were taken out and checked for the growth of bacterial colonies.

ix. Bacterial colonies were count by using bacterial colony counter.

x. Determine the number of respective microorganisms per milliliter of original culture

(gram of soil) as follows:

Microorganisms per gram of soil = count per plate/ dilution used

For example, if 200 colonies were present on the 10^{-7} plate, the calculation would be

Microorganisms per gram of soil = $200/10^{-7}$

$=2.0 \times 10^9$ colonies/gram

Result:- The bacteria has been isolated from the soil sample and its colonies number increases/decreases with dilutions made.

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Experiment No:-9

Aim: To determine the microbial flora in laboratory tap water and drinking water(college and hostel).

Reference:- Michael J Pelczar, E.C.S Chan, Noel R Krieg, Microbiology an application based approach, published by MC Graw Hill, New Delhi, third reprint 2011, pg. no. 820-823

Requirements: nutrient broth media, test tubes, glucose/dextrose,

Theory

Regarding the microbial flora in drinking water, it comprises various coli forms group of bacteria i.e. *E .coli,* enterobacter, salmonells, schigella, klebsiella,etc. Bacteria present other than coli forms are streptococci, *S. bovis, etc.* Thus the presence of these bacterial species in water is the evidence of faecal pollution of animal or human and when discharged, may enter a body of water that ultimately serve either as source of drinking or washing.

Procedure

1. Sterile Nutrient broth media as prepared in experiment was used .
2. These nutrient media was subdivided into 4 sterilized test tubes, each test tube was then added with 500mg of glucose/dextrose and shaked well.
3. 0.3 ml of water was collected from listed sources and was poured in to three test tubes, leaving fourth test tube as a control tube.
4. All the test tubes were plugged with cotton and aluminium foil paper and kept in the incubator at 37^0C temperature for 24 hours.
5. After 24hours, removed all test tubes from the incubator and checked for the growth of microorganism by comparing with the control testtube.

Result: After performing above experiment it was found that microbial flora was present/absent in…..

Experiment 10

Aim: To test the sterility of a pharmaceutical product as per I.P.

References:-

Requirements:- Fluid thioglycolate medium, alternative thioglycolate medium, soyabean-casein digest medium, *Bacillus subtilis*, *Candida albicans*, *Bacteroides vulgates*, 0.45µm membrane filter.

Fluid A:- Prepared by dissolving 1gm peptone in water to make 1 liter, clarified by filtration, adjusted to pH 7.1±0.2 and sterilized at 121˚C for 20 minutes.

Fluid B:- It is fluid A to which 1ml Tween 80/L has been added.

Theory:- The test for sterility are intended for detecting the presence of viable forms of microorganism in or on pharmaceutical preparations.

The test should be performed in such condition that there should not be accidental contamination during test. Working place should be monitored regularly.

Test for sterility is based on the principle that “if microorganisms are placed in a medium which provides a nutritive material and water, and kept at a favourable temperature, the organism will grow and their presence can be indicated by turbidity in the originally clear medium”.

Test Procedure: I.P. recommends tweo methods viz.

Method A- Membrane Filtration

Method B- Direct Inoculation

Method A:- Membrane Filtration:- The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily solvent, provided these solvents do not have antimicrobial effect in the condition of the test.

Apparatus- Apparatus consisting of a closed reservoir and a receptor between which a properly supported membrane of appropriate porosity is placed. The membrane has a nomial pore size not greater than 0.45µm and diameter 47mm. the apparatus is assembled and whole unit is sterilized.

Procedure:-

Prepared each membrane by aseptically transferring a small quantity (sufficient to moisten the membrane) of fluid A on the membrane and filtered it.

Transfer the contents of the container or pharmaceutical product to be tested to the membrane. Filer immediately.

Note:- if the product to be tested has antimicrobial properties, wash membrane by filtering through it no less than 3 successive quantities of approximately 100ml sterile fluid A.

After filteration aseptically remove the membrane from the holder and cut the membrane in to two equal parts.

Immerse the membrane in 100ml of soyabean casein digest medium and incubate at 20-25˚C for not less that 7days and similarly the second membrane part in sodium thioglycolate medium (30-35˚C)

Method B:- Direct Inoculation-

Transfer aseptically specified volume of material from each container to culture medium. Mixed the liquid with medium.

The inoculated medium is incubated for not less than 14 days at 30-35˚C (fluid thioglycolate medium) and at 20-25˚C (soyabean casein digest medium).

Note:- If the product to be examined has antimicrobial activity, carryout the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture media.

Dr. Arun K Mishra *June 28, 2022 at 9:34AM*

Excellent

REPLY

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