**Preparation of basic liquid Medium (broth) for routine Cultivation of Bacteria**

Bacteria are often cultivated in liquid broth (media lacking agar)

**Materials:** Peptone 5g, Beef extract 3 g, distilled water 1 I , 0.1 N HCl , 0.1 N NaOH,

pressure cooker, 1 L beaker measuring cylinder, non-absorbent cotton, test tube and

pH paper.

**Procedure:** Take the weighed amounts of peptone and beef extract and mix in 50 ml of

distilled water and heat it is dissolve the contents. Add more distilled water to make it to 1

L. Adjust the pH to 7 using pH papers by adding either acid or alkali as the case may be.

Take this into the test rube and apply cotton plug, sterilize at 15 Ibs pressure for 15 mts

in pressure cooker. Allow the pressure cooker to cool, remove the nutrient broth tubes

and store at room temp and cover with butter paper.

**Preparation of Basic Solid Medium**

Liquid broth media containing nutrients are usually solidified by the addition of agar.

**Eg.** Potato Dextrose agar medium, Nutrient agar medium.

**A) Preparation of Potato Dextrose Agar Medium:** Used in isolation and maintenance

of common fungi.

**Materials:** Peeled potatoes - 200g, Dextrose - 20 g. Agar - 20 g, Distilled water 1 L,

beaker 1L, 250 ml conical flasks, knife, muslin cloth, measuring cylinder, cotton nonabsorbent,

pressure cooker.

**Procedure**

1. Take 500 ml of distilled water in 1L beaker and add 200g of peeled and sliced

potato boil the potatoes till they become soft.

2. Filter the contents of the beaker through muslin cloth and squeeze out all liquid

3. Add the dextrose dissolved in water to this extract.

4. Adjust the pH of medium to 6 to 6.5 using 0.1 N HCl or 0.1N NaOH as the ease

maybe

5. Add the dissolved agar to dextrose-potato extract and make the volume to 1lt

and dispense 200ml each to 5 conical flask and plug with non absorbent cotton.

Sterilise the flasks at 15 Ibs pressure for 15 mts in a pressure cooker.

Allow the pressure cooker to cool, "Remove the conical flask and store at room

temperature. Allow the flask to cool until the flask can be held by hand.

7. Prepare agar plate by pouring the media into Petri-dish quickly. Using aseptic

condition, allow the media in Petri-dish to solidify to produce the agar plate.

**B. Preparation of Nutrient Agar Medium:** Used for the maintenance and isolation of bacteria.

**Materials:** peptone - 5g, beef extract - 3g, Agar - 20g, distilled water -1lt, Petri-dish, 1lt

beaker, 250 ml conical flasks, measuring cylinder, non absorbent cotton, pressure

cooker and hot plate.

**Procedure**

1. Dissolve the weighed amounts of peptone and beef extract into 500 ml of water.

2. Heat and dissolve the chemicals and adjust the pH of medium to 7 by adding

0.1N HCl or 0.IN NaOH.

3. Weigh 20g agar and dissolve in 500 ml of distilled water in another beaker

4. Mix the dissolved agar with chemical solution and make up the vol. to 1lt.

5. Dispense 200 ml each into 5 conical flasks.

6. Plug the flask with non absorbent cotton and sterilise at 15 Ibs pressure for 15

mts in a Pressure cooker.

7. Allow the cooker to cool, remove the conical flask and store at room temp, or

8. Allow the flask to sufficiently cool and prepare agar plates by pouring media into

Petri-dish under aseptic condition; allow the media with Petri-dish to solidify.

**Precautions**

1. Don't pour the media over 2/3 of flask capacity.

2. Cotton plug must be loose whale autoclaving.

3. Don't pour media to Petri-plate when the medium is too hot since it produce

condensation of water on underside of Petri plate lid and thus can fall on to agar

surface and may lead to contamination and spreading of colonies.

4. Pour medium quickly to avoid contamination by air-pores and close lid down as

soon as possible.

5. Perform the pouring of medium in inoculation chamber fitted with U. V. lamp with

filtered air.

6. Pouring should be performed near the flame.

**Observation**

After sterilization of medium observe the medium in conical flask and plate for

solidification.

After incubation period of 24-48 hrs for nutrient agar medium and 7 days for PDA

observe the growth of any microbe on the surface of the medium.

**Exercise**

1. Prepare 500 ml nutrient agar medium.

2. Prepare 500 ml potato dextrose agar medium