Microbiology Laboratory #3 - Preparation of Microbiological Growth Medium.

Any medium for the cultivation of bacteria must provide certain basic nutritional requirements including:

(1) a carbon source that may also serve as an energy source
(2) water
(3) a nitrogen source
(4) phosphate and sulfur sources
(5) various mineral nutrients, such as iron and magnesium

Some bacteria are capable of growth on a medium consisting of a single carbon source, such as the carbohydrate glucose, a simple nitrogen source, such as ammonium salts, and inorganic salts, such as phosphates. This kind of medium is termed defined or synthetic because its exact chemical composition is known.

For routine laboratory work, complex media are usually employed. In these media, the basic nutrients are provided by complex nutrients, such as plant, animal, or yeast extracts in which the exact composition is not known. For example, beef extract and peptones (hydrolyzed protein) are the basic ingredients of nutrient agar. These materials supply a variety of carbon sources, nitrogen compounds in the form of amino acids, and a mixture of cofactors, such as vitamins. This basic medium can be enriched to support the growth of more fastidious types of bacteria by the addition of carbohydrate sources, yeast extract, and materials such as plasma or blood, which provide a variety of complex nutritional factors. The pH may have to be adjusted to permit microbial growth.

A broth medium is one in which the components are simply dissolved in water. The addition of agar (a complex carbohydrate extracted from seaweed) results in a solid medium. Agar is an ideal solidifying agent for microbiological media because of its melting properties and because it has *no nutritive value* for the vast majority of bacteria. Solid agar melts at 90-100°C; liquid agar solidifies at about 42°C. Many microbiological media contain 1.5% agar.

Some types of media contain ingredients that inhibit the growth of some organisms and stimulate the growth of others. Such **selective media** are used to identify and quantify specific microbes within a mixed population. In contrast, **differential media** do not necessarily inhibit the growth of organisms, but rather provide a mechanism to distinguish different types of microbes. Many differential media, such as the carbohydrate broths we will make today, contain pH indicators to reveal the production of acidic or basic waste products.

Because microorganisms are ubiquitously distributed in the environment, bacteria are introduced from many sources such as glassware, dry medium components, air, and so on. These bacteria would eventually grow and flourish if the medium were not sterilized, that is, if these unwanted microorganisms were not destroyed. Sterilization procedures eliminate all viable microorganisms from materials. Culture plates, test tubes, flasks, pipettes, transfer loops, and media must be free of viable microorganisms before they can be used for establishing pure cultures of microorganisms. Culture vessels must be sealed or capped with sterile plugs to prevent contamination. There are various ways of sterilizing the liquids, containers, and instruments used in pure culture procedures; these include exposure to elevated temperatures or certain types of radiation to kill microorganisms and filtration to remove microorganisms from a solution.

Media preparation for the microbiology laboratory involves the use of an autoclave for sterilization, which permits exposure to high temperatures for a specified period of time. Generally, a temperature of 121°C (achieved by using steam at 15 lb/in^2) for 20 minutes is used to heat-sterilize bacteriological media. Autoclaves have different exhaust modes (cooling times) for liquid, solid, and wrapped goods. Much of the time spent in preparation for the bacteriology laboratory involves cooking the media for growing bacteria; that is, mixing and sterilizing the growth media in suitable containers

A. Preparation of Tryptic Soy Broth and Agar, a general medium.

1. Always begin work by disinfecting your work area. Spray disinfectant on the benchtop and wipe off with paper towels.

2. Add 300 mL deionized water to a 500 mL flask. Label the flask “TSA”.

3. Add 4.5 g tryptone, 1.5 g soytone, 1.5 g NaCl to the 300 mL dH_2O. Swirl or stir with a glass stirring rod until dissolved.

4. Use a 50 mL graduated centrifuge tube to measure 50 mL of this solution to distribute into each of two 125 mL flasks.

5. Label the flasks with your initials and “TSB”. Cover the flasks with aluminum foil, place a small piece of autoclave tape on the top. You will use these flasks of TSB for your lab practical at the end of the semester!

6. Add 3.0 g agar to the remaining 200 mL of solution in the 500 mL flask. Cover the flask with aluminum foil, place a small piece if autoclave tape on the top.

7. Label 8 empty, sterile, petri plates with “TSA”, your initials and the date.

8. Obtain a 500 mL flask containing 200 mL TSA fresh from the autoclave.

9. When the flask has cooled to the point that it does not burn your hands, fill 8 petri plates as demonstrated by your instructor or an assistant. Immediately rinse flask with hot tap water and place in dirty glassware tub.

10. Place TSA and TSB flasks from steps 6 and 7 into autoclave pan on front bench.

B. Preparation of Carbohydrate Broths, a group of differential media.

1. Add 150 mL dH_2O to a 200 mL beaker.

2. Add 2.4 g phenol red broth base to the 150 mL dH_2O. Stir with a glass stirring rod until dissolved.

3. Depending on the carbohydrate assigned to your group, clearly label (50) 13x100 tubes with one of the following letters
   C – cellobiose
   G – glucose
   S – sucrose
   L – lactose
   X – xylose
   M – mannitol

4. Add 1 g of the appropriate carbohydrate to the dissolved phenol red broth base. Once dissolved, check the pH using pH paper or a pH meter and adjust to 7.2 with a few drops of 0.1 M NaOH if necessary. Distribute the medium into tubes in 3 mL aliquots.

5. Place an inverted durham tube into the tube of medium, place a cap over the tube, combine in a rack with your neighbor, who also used the same carbohydrate and place the rack in the pan "to be autoclaved".

6. Always conclude work by disinfecting your work area. Spray disinfectant on the benchtop and wipe off with paper towels.

7. While we are not preparing R2A medium due to the number of components and the small amount of each, the recipe is listed below for your reference. This low nutrient medium supports the growth of freshwater organisms particularly well.

830. R2A MEDIUM

Yeast extract	0.50	g
Proteose Peptone (Difco no. 3)	0.50	g
Casamino acids	0.50	g
Glucose	0.50	g
Soluble starch	0.50	g
Na-pyruvate	0.30	g
K_2HPO_4	0.30	g
$MgSO_4 \times 7\ H_2O$	0.05	g
Agar	15.00	g
Distilled water	1000.00	ml

Final pH 7.2; adjust with crystalline K_2HPO_4 or KH_2PO_4 before adding agar. Add agar, heat medium to boiling to dissolve agar, and autoclave for 15 min at 121°C.