

EXERCISE # 5**PREPARATION OF GENERAL BACTERIOLOGICAL MEDIUM**

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and contains many nutrients needed for the bacterial growth. It consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms as well as enumeration of organisms in water, sewage, dairy products, feces and other materials.

COMPOSITION OF NUTRIENT AGAR

Beef Extract.....3.0 g
Peptone.....5.0 g
Agar.....15.0 g
Distilled Water.....1000 mL
Final pH 6.8 ± 0.2 .

Composition of Nutrient Broth: Nutrient broth contains same ingredients except agar.

CHARACTERISTICS OF THE COMPONENTS USED IN NUTRIENT AGAR/BROTH

- **Beef extract** is an aqueous extract of lean beef tissues. It contains water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water soluble vitamins, and salts.
- **Peptone** is made by digesting proteinaceous materials e.g., meat, casein, gelatin, using acids or enzymes. Peptone is the principal source of organic nitrogen and may contain carbohydrates or vitamins. Depending up on the nature of protein and method of digestion, peptones differ in their constituents, differing in their ability to support the growth of bacteria.
- **Agar** is a complex carbohydrate obtained from certain marine algae. It is used as a solidifying agent for media and does not have any nutritive value. Agar gels when the temperature of media reaches 45°C and melts when the temperature reaches 95 °C.

PREPARATION OF NUTRIENT AGAR

Nutrient agar and broth are available commercially in powdered (free-flowing, homogeneous) form.

1. Dissolve the dehydrated medium in the appropriate volume of distilled water i.e., 23 gm dehydrated nutrient agar (*see the manufacturer instruction*) in 1000 ml distilled water.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
3. Sterilized the medium by autoclaving (121°C for 15 min)
4. Dispense the medium in to tubes or plates. Left the agar medium to solidify and store.
5. Determine the pH of the medium (pH 6.8 ± 0.2) with a pH meter and adjust if necessary.

USES OF NUTRIENT AGAR/BROTH

1. For the enumeration of organisms in water, sewage, dairy products, feces and other materials.
2. For the cultivation and maintenance of non-fastidious species.

QUALITY CONTROL

1. The color of prepared Nutrient Agar will be light amber, very slightly to slightly opalescent
2. pH of the prepared media should be 6.8 ± 0.2 .
3. Media should be checked for their performance using stable, typical control cultures. When the prepared media are inoculated with *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and incubate at $35 \pm 2^\circ\text{C}$ for 18-48 hours, media should show good growth of these organisms.

QUESTIONS

1. Name the general purpose media for bacteria?
2. What is the recipe for 500mL nutrient agar media?

What is the identification of proper media?