

# Water Culture Procedure

## **Purpose:**

To determine the gram-negative bacteria count in a 100ml water sample.

## **Special Materials:**

- Filter membrane- sterile, individually-wrapped, white, 47mm diameter, 0.45um pores
- Buchner funnel- glazed porcelain, 50mm inside diameter, autoclaved
- Vacuum filtering flask
- One-holed rubber stopper- size corresponding to the mouth of the flask, and hole diameter corresponding to the stem of the Buchner funnel
- Hand-operated vacuum pump- with tubing to fit the arm of the flask and having a vacuum release valve

## **Plating:**

1. Connect the vacuum pump tubing to the arm of the flask and insert the rubber stopper into the mouth of the flask.
2. Unwrap the autoclaved Buchner funnel and place its stem snugly into the hole of the rubber stopper in the mouth of the flask, being careful to not contaminate the inside surfaces of the funnel.
3. Sterilize the tip of a pair of forceps by rinsing with alcohol then flaming with a lighter.
4. Carefully open the wrapper on a sterile filter membrane and use the sterilized forceps to place the membrane inside the Buchner funnel.
5. Shake the water sample, then carefully pour it into a sterile 100ml sample cup to measure out exactly 100ml of water.

6. Slowly pour the 100ml of water into the Bucher funnel while operating the hand vacuum pump to create suction, pulling the water through the filter membrane.
7. When all 100 ml of water appears to have passed through the membrane and water is no longer dripping into the bottom of the flask, open the release valve on the vacuum pump to release the suction.
8. Sterilize the tip of the forceps again, then use the forceps to carefully remove the filter membrane from the Buchner funnel and place it on the center of a MacConkey plate. Use the forceps to gently push the membrane into contact with the surface of the agar, so there are no trapped air bubbles.
9. Incubate at 37°C for 18-24 hours with plates inverted.

### **Reading:**

1. After incubation, identify any colonies growing on the membrane as follows:
  - Pseudomonas: translucent, weak catalase reaction, oxidase-positive
  - Serratia: translucent, vigorous catalase reaction, oxidase-negative
  - Coliforms: pinkish, opaque, weak catalase reaction, oxidase-negative
2. Count the number of each type of colony and divide by 100 to determine the CFU/ml of water.