SOP-M003

Serial Dilutions for Bacteria

Objective: To dilute a colony of bacteria for plating.

Procedure #1:

Note: Be sure you have labeled your dish with the following information: Bacteria Type, Date, and your Initial.

1. Put 4mls of PBS in sterile glass tubes with the caps slightly loosened. Autoclave on the liquid program. You will be using 4 tubes for each bacterium to make the serial dilution.

2. Once the tubes are cooled, remove one colony from your petri dish or test tube using a disposable inoculating Loop. (VWR cat#90001-100 BD Difco Blue 10ul) Not touching the sides of the glass tube, put the colony in the first tube of PBS. Swish back and forth and up and down until the colony is off the loop. IT IS BEST TO RUB THE COLONY ONTO THE SIDE OF THE GLASS TUBE JUST ABOVE THE LIQUID LINE – THIS REMOVES THE ORGANISMS FROM THE LOOP.

Move the loop up and down through the deposit to mix it into the liquid. The goal is to disaggregate the sticky colony into single cell suspension.

Place the cap back on tightly and gently shake the tube against your hand until the colony is mixed well with the PBS.

3. Using a 200ul pipette, remove **100ul** of diluted bacteria solution from tube one and place it into tube two. Put the cap on and gently shake this tube against your hand.

4. Repeat #3 going from tube three to tube four.

5. Now you have your last dilution tube ready for plating. Be sure there isn't any condensation on the lids of the petri dishes. This time remove only 50ul of solution from tube four and place it in the center of your Agar.

6. With an L-shape spreader (RPI Corp. cat#247660 Lazy-L Spreader, disposable 10/pouch) gently glide it on the Agar to spread the bacteria dilutions. Place the lid on the side of the petri dish so the sample is able to dry.

7. Once dry, place the lid on the dish and place it in the 37°C incubator with the Agar side up. Check daily to see how your colonies are growing. Some grow faster than others.

Procedure #2: (modified May 19, 2015)

Note: Be sure you have labeled your dish with the following information: Bacteria Type, Date, and your Initial.

1. Put 4mls of PBS in sterile glass tubes with the caps slightly loosened. Autoclave on the liquid program. You will be using 4 tubes for each bacterium to make the serial dilution.

2. Once the tubes are cooled, remove one colony from your petri dish or test tube using a disposable inoculating Loop. (VWR cat#90001-100 BD Difco Blue 10ul) Not touching the sides of the glass tube, put the colony in the first tube of PBS. Swish back and forth and up and down until the colony is off the loop. Place the cap back on tightly and gently shake the tube against your hand until the colony is mixed well with the PBS.

3. Using a 100ul pipette, remove **25ul** of diluted bacteria solution from tube one and place it into tube two. Put the cap on and gently shake this tube against your hand.

4. Now you have your last dilution tube ready for plating. Be sure there isn't any condensation on the lids of the petri dishes. This time remove only 50ul of solution from tube two and place it in the center of your Agar.

6. With an L-shape spreader (RPI Corp. cat#247660 Lazy-L Spreader, disposable 10/pouch) gently glide it on the Agar to spread the bacteria dilutions. Place the lid on the side of the petri dish so the sample is able to dry.

7. Once dry, place the lid on the dish and place it in the 37°C incubator with the Agar side up. Check daily to see how your colonies are growing. Some grow faster than others.

Created by: J.P. Robinson

Verified by:_____ Date: May 19, 2015 _____

Print Name Sign Name