

SOP-P074

(Ray's) Drip Plate Serial Dilutions for Bacteria

Objective: To dilute a colony of bacteria for plating.

Procedure:

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

1. 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 side of the polystyrene tube put the colony in the tube containing 4mls of sterile Tryptiscase Soy Broth (VWR cat#211043). Swich 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 broth.
2. Put 4.5mls of Hanks' (Autoclaved) Solution (Hanks'Balanced Salts, Modified Sigma Cat# H4891-1L) in sterile 14ml Polystyrene Round-Bottom Tubes (Becton Dickinson, Falcon Cat#352057). You will be using 6 tubes for each bacterium to make the serial dilution. Labeled tubes #1 - #6.
3. Using a 1000ul pipette, remove **500ul** of diluted bacteria solution from tube containing the sample in Broth. Place it into tube One. Put the cap on and gently shake this tube against your hand.
4. Repeat step #3 going from tube one to tube two. Then from tube two to tube three and so on until you reach your last tube six.
5. Now you have your last dilution tube ready for plating. **Be sure that your pre-poured plates are dry.**
6. Place your pre-poured plates at a 45° angle. Remove 10uls of tube #6. Starting from left to right, put all of the 10uls at the top of the plate and let it drip down to the bottom. Repeat this step 12 times, or until you reach the right side of the plate. Replace the lid on the plate and stand it on it's side until all of the drips fall to the bottom of the plate. Once the drips are completed, place the plate Agar side up in the hood for approximately 16 hours.
7. The following morning, put the plates in the 37°C incubator with the Agar side up for 2 hours before you start scanning. Once scanning has started, scan once every 60 minutes. Stop scanning when you have reached the maximum growth you're looking for.

Created by: Cheryl Holdman

Verified by: _____ **Date:** 8/10/2011 _____

Print Name

Sign Name