Lab 2 – Pouring bacterial plates

Background:
Read pages 245 – 246 (Setting Up), 249-251 (Growth and maintenance), 253-254 (Protocol for pouring plates), and 257-258 (Antibiotics) in *At the Bench*.

Purpose:
To practice proper laboratory technique regarding streaking bacteria and preparing and pouring agar plates.

Please read the entire lab, background information, and perform calculations (Part B steps 4 & 6) before starting. The materials you are using today are time-sensitive so you cannot afford to stop mid-procedure.

Part A – Streaking Bacteria for Lab #3
Protocol: You will need 1 LB/ agar plate total per two people. Always keep the agar plate on top of the other plate unless you are using it.

1. Label the plate with the agar with your name, the date, and the bug you are to steak (*E. coli*).
2. Flame the loop over the Bunsen burner until red.
3. Take the tube of bacterial culture with your left hand, swirl to resuspend, and remove the cap with the last two fingers of the right hand. Flame the open tube by passing it for 1 second through the flame.
4. Insert the loop into the tube, without touching the sides of the tube. Insert the loop deep enough to only submerge the circle part.
5. Pass the tube through the flame again, replace the lid, an return the tube to the rack.
6. Lift the agar plate with your left hand and hold it agar side up. Streak the plate with you the inoculation loop as described by the instructor.
7. Return the agar plate to its other half and place the petri dish in the 37 degree incubator
8. Flame the loop again to sterilize it.

Part B – Agar plate preparation for Lab #3
Protocol: You will need 4 plates total per two people.
(The book gives more details about pouring plates and you are expected to follow the instructions on technique.)

1. Label four sterile petri dishes on the bottom of the dish taking care not open the lid and decontaminate the dishes. Label *one* dish LB, *two* dishes LB/AMP, and the *last* dish LB/AMP/ARA. Include your initials and the date.
2. Place your media bottle in the 50ºC in water bath containing at about 120 mL of LB/agar for each person in your group until you are ready to pour it. The LB has been autoclaved with the agar to completely melt the agar.

3. Pour the liquid into the plate labeled LB near the “Cone of Sterility” (Note: the agar solidifies quickly at room temperature so you need to move quickly.)
4. Add 500X ampicillin to 1X concentration (stored in the ice box on the teacher station) when not too hot → include under calculations section. Remember your volume is 120 mL less 20mL you poured already.
5. Pour agar with ampicillin into two plates labeled LB/AMP.
6. Add 50X → 1X concentration ARA to the bottle of agar with ampicillin (assume you removed 60mL from your initial volume of 120 mL). Put in calculations section. Pour one plate into the LB/AGAR/AMP plate.
7. Allow plates to cool and solidify on bench near the “Cone of Sterility” (under the flame).
8. Stack plates upside down and wrap as a set of four with parafilm to deter contamination.

Data/Results: This section should contain all your calculations from steps 4 and 6 with all units clearly labeled.

Conclusion: As there is not conclusion for this lab, answer the questions below for full credit.

Questions:

1. What did you make in the lab?
2. Why do scientists use antibiotics in the lab?
3. What is the working concentration in terms of µg/mL of ampicillin that the book recommends?
4. What are the four phases of a normal growth curve of bacteria?
5. Describe the level of biosafety/containment that is necessary for the bacteria used in this laboratory exercise?