

1. Gather equipment: sterilized swabs and agar petri dish and gloves.
2. Wash hands with hand disinfectant
3. prepare work area: place paper towels on counter; pour surface disinfectant onto them until just wet.
4. light the bunsen burner
5. select the culture to be transferred
6. select a tube of broth, an agar slant, and an agar plate to be inoculated.
7. label each appropriately with the following: name of culture used, your name, and date. Label bottom of the agar plate.
10. Inoculate the agar plate:
  - a. place the petri dish containing the agar plate on the counter
  - b. hold the tube containing the bacterial growth in the left hand and the inoculating loop in the right hand.
  - c. flame the loop and allow to cool
  - d. remove the cap from the tube, briefly flame the mouth of the tube, and use the ~~loop~~ loop to obtain a portion of a colony of bacterial culture.
  - e. withdraw the loop, flame the mouth of the tube, replace the cap and place the test tube on the rack.
  - f. open the petri dish lid with the left hand just enough (2 1/2 inches) to allow the entrance of the loop.
  - g. streak one quadrant by spreading the organisms, making six to eight streaks.
  - h. flame the loop and cool it
  - i. turn the petri dish one quarter turn.
  - j. streak the second quadrant making six to eight streaks, entering the previously

- 9. streaked quadrant two to three times.
- 10. repeat steps i-j for the third quadrant
- 11. begin the streaks in the fourth quadrant as in the other quadrants; continue making the streaks, decreasing the width and increasing the distance between the streaks to form a "tornado-like" pattern.
- 12. turn off bunsen burner
- 13. place broth + slant tubes into a test tube rack in a 37°C incubator. (leave caps slightly loosened). Place the agar plate upside down in the 37°C incubator
- 14. clean & equipment and return to proper storage
- 15. clean work area with surface disinfectant
- 16. wash hands with hand disinfectant
- 17. check the broth, slant, and agar plate for bacterial growth after overnight incubation. The broth should be cloudy, indicating growth of organisms. The slant should have a zigzag formation of bacterial growth on the surface. The agar plate should have bacteria growing in all four quadrants with some isolated colonies in the third and fourth quadrants.
- 18. Be sure to observe colonies.

Colony Morphology: Part A

- Quadrant 1: yellow, pink, + white irregular, circular, raised, entire.
- Quadrant 2: circular, irregular raised, undulate. white
- Quadrant 3: pink, white, yellow circular, raised, irregular, filamentous undulate.
- Quadrant 4: circular, raised, filiform. white

Part B: Quad I (very little colonies)  
shape = coccus and bacillus  
grouping = streptococcus

Overgrowth.

KB 2/24/06  
↓

Conclusion: I had many different types of bacteria. There were little bacteria in some quad's.

KB 2/28/06  
Conclusion: I didn't have a lot of colonies. The ones I did have grew together with bacteria all around it.