Name _____

Microbiology
Streaking and Isolation Procedure

Materials Required:

- Bacteriological Loop and Incinerator
- Trypticase soy agar (TSA), 3 plates per student
- Specimens: Trypticase soy agar (TSA) pure cultures; mixed broth culture

Clinical Purpose: (2 pts)

Streaking for isolation technique:

1. **Inoculation:** This step may be done with a sterile loop, needle, pipet, swab, or forceps as required by the specimen type. All instruments and agar plates, media, tubes, etc, must be sterile. Sterile technique is required during all steps to prevent culture contamination. Label the media side of the plate with a permanent marker. Include culture type, date, and your initials. Using a sterilized loop, place a small amount of inoculum into onto the media as directed.

   Agar: Dispense the inoculum onto the agar at the top of the dish
   Broth: Take a loopful of broth and place onto the agar at the top of the dish. Do not flame at the end of each quadrant.

2. **Streaking** (four quadrant method)**: Carefully observe the demonstration. Then streak two of your plates for isolation.**

   a. Using gentle pressure, use a sterilized loop to evenly spread the inoculum over the top ~1/6 of the plate (zone 1). Keep the loop flat and at a low angle. This should help reduce "digging" into the media. Flame your loop to sterilize.
   b. Rotate the plate 1/3 of its circumference. Begin the streaking process by placing the loop in zone one and drawing the inoculum down into the zone two. Without removing the loop from the media move the loop back into zone one. Repeat this step three times. Flame loop.
   c. Now streak the inoculum through this zone. Always draw the loop away from the last zone. Do not overlap your streak lines. Do not reenter the prior zone.
   d. Rotate the plate and repeat the process used in zone two in zones three and zone four. Ensure that you use the entire surface of the media.

3. **Isolation from broth media:** Mixed culture TSB to TSA: Proceed as described above except do not flame after streaking quadrants one and two. Label your plate with the color of the tape on the tube.

Incubation: Place all TSA plates into the incubator rack provided. Incubate 18-24 hours at 35^0C.

DAY 2

1. Examine each TSA plate separately. Describe the colonies. Are they white, gray, yellow, red? Are they tiny, small, medium, or large? Are there different colony types? Record your observations.

	Isolation (Y/N)	Colony Description
Mixed TSB broth tape ( )		
Pure TSA plate 1		
Pure TSA plate 2		

1. On the reverse of this page draw a circle approximately ten centimeters (3 ½ inches) in diameter. Using a pencil, perform a quadrant streak plate method in this circle. Be sure to use the entire surface of the plate. (1 pts)

2. Examine all the streak plates. Did you achieve isolation? Were the first three streaks near the edge edges of the plate? Did any streaks intersect streaks they shouldn't have? Was the entire surface of the plate used? (2 pts)

3. Hypothetically, if you observed more growth in quadrant 4 than in quadrant 3, how would you explain this result? (3 pts)

4. Most colonies on streak plates grow from isolated colony forming units (CFUs). Sometimes, a colony can be a mixture of two or more different organism. If a culture started from this colony (Initially, you thought it was pure.) correct identification would be impossible because each organism would alter the test results. How could you verify the purity of the culture? If you found the culture to be a mixture, what could you do to purify it? (3 pts)