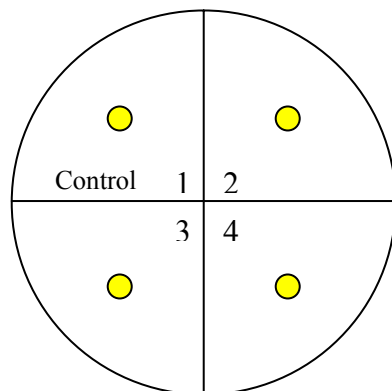


## Bacterial Spread Technique

1. Using a marker, label the bottom of the Petri dishes with ALL essential information, as Petri dishes basically all look the same. Be sure to include your period – group number, level of IV, and/or any other pertinent information. In your lab notes, label 1-4 and corresponding antibiotic or antiseptic.
2. Obtain a stock culture of bacteria. For spread plates, cultures in a broth (liquid) are preferred. Always hold and store the stock culture in an upright position to prevent the bacteria from coming in contact with the tube's cap.
3. Light a Bunsen burner as previously described.
4. Open the culture of bacteria, being careful to not lay the top of the test tube down on the counter. Pass the mouth of the tube through the flame of the Bunsen burner two or three times to sterilize it. Insert a sterile pipet into the tube, and carefully draw approximately .5 ml of broth containing bacteria. Once again pass the mouth of the tube through the flame of the Bunsen burner two or three times to sterilize it. Replace the cap and return the tube to the test tube rack. **NOTE: Do NOT put the pipet containing the bacterial broth down while you are returning the tube to the test tube rack.** Turn off the Bunsen burner.
5. Open one Petri dish at a 45-degree angle. **NOTE: Do not remove the lid completely.** Release one (.5) mL of the broth on the surface of the agar. Be careful not to release more than .5 mL of the broth onto the surface of the agar. Close the Petri dish, and set it aside.
6. Place the pipet into the designated container provided by your instructor. Do **NOT** set the pipet down on the lab bench at any time.
7. Open the Petri dish at a 45-degree angle. Place a sterile spreader bar(See #8) in the bacteria in the center of the dish. Carefully spread the bacteria evenly over the surface of the agar by turning the Petri dish in circles until the bacterial broth completely covers the agar. Immediately close the lid of the Petri dish. Tape the dish closed. Wait a few minutes for the broth to dry, and then turn the Petri dish upside down, and set it aside.
8. If a spreader bar is being used, it must be sterilized before being used to spread the bacteria. Place the bar in a beaker of alcohol (ethyl). Remove the bar from the alcohol, and place it in the flame of the Bunsen burner. Remove the bar from the flame, allowing the alcohol to burn off. **DO NOT INSERT A HOT SPREADER BAR BACK INTO THE CONTAINER OF ALCOHOL.** Allow the bar to cool in the air for several seconds. Make sure the bar is cool by opening the Petri dish at a 45-degree angle, and touching it to an area of the dish where there is NO bacteria. If it melts the agar, allow it to cool longer. When it has cooled sufficiently, open the Petri dish at a 45-degree angle, and spread the bacteria over the entire surface of the Petri dish. **Remember:NEVER completely remove the lid of the Petri dish.**



Ex. Petri dish with quadrants drawn for sterile blanks with test substance. Measure zone of inhibition around each disc in mm.