Sterile Technique

It is very important in microbiology to work with pure cultures. Unfortunately this is difficult. The world around us is covered with microorganisms. Microorganisms are even carried on dust particles in the air. In order to protect sterile broth, plates, slants and pure cultures from the microbes all around us, we must practice sterile (aseptic) technique. This simply means that sterile surfaces or sterile media must be protected from contamination by microbes in the air or residing on non-sterile surfaces. A simple example of the problem is that a sterile petri plate can become contaminated with bacteria when the lid is removed. In sterile technique, only sterile surfaces touch other sterile surfaces and exposure to the air is kept to a minimum.

In the classroom you often need to practice sterile technique when you inoculate a pure culture of a microorganism into fresh medium. Sometimes this is a transfer to a tube of liquid broth and at other times it is a transfer to a petri plate containing agar. While there are other circumstances that require sterile technique, these are the most common and they will be described in more detail on the pages that follow.

Transferring a Broth Culture to Fresh Broth

Materials

- Bunsen burner
- inoculating loop
- marking pen
- broth culture of microorganism to be transferred (tube A)
- sterile broth in a culture tube with a loose-fitting cap (tube B)

Procedure

1. Light the Bunsen burner.
2. Place the culture you wish to transfer (tube A) near the tube of sterile broth that you will inoculate (tube B).
3. Label the tube B with the name of the microorganism and the date.
4. Hold the inoculating loop with your thumb and first two fingers. Heat the inoculating loop in the Bunsen burner until it is red hot. Heat several inches of the loop, since that much of it will contact the inside of the tubes. Allow the loop to cool for a few seconds while you hold it in your hand. Do not put it down or allow the loop to touch any surface after it is sterile.
5. While continuing to hold the inoculating loop with your thumb and first two fingers, pick up tube A in your left hand and remove the cap with the last two fingers of your right hand. Keep the cap in your right hand and do not allow it to touch any surface.
6. Using your left hand, draw the open top of tube A gently through the flame of the Bunsen burner. Do not hold it in the flame for more than a second.
7. Place the sterile loop into tube A containing the culture until the loop is in the liquid. Carefully draw the loop out of the tube and continue to hold it in your hand.
8. Draw the open top of tube A gently through the flame of the Bunsen burner and then replace the cap on the culture tube A. Replace tube A in its rack.
9. While still holding the loop with the inoculum in your right hand, pick up the sterile tube of broth (tube B) with your left hand. Remove the cap with the last two fingers of your right hand. Keep the cap in your right hand and do not allow it to touch any surface.
10. Draw the open top of the sterile tube B gently through the flame. Do not hold it in the flame for more than a second.
11. Place the loop containing the droplet of culture into the tube B and gently swirl it to transfer the microorganisms to the sterile broth.
12. Remove the loop from the broth and continue to hold it n your hand.
13. Draw the open top of the sterile tube B gently through the flame and then replace the cap, which should still be in your right hand, on tube B. Place tube B back in the test tube rack.
14. Heat the inoculating loop in the Bunsen burner until it is red hot. You can now place it on the bench or in a rack.
15. At no time should the inoculating loop, the top of the tubes or the inside of the cap have touched any non-sterile surface (especially your fingers or hand).

Streaking a Broth Culture to a Fresh Petri Plate

Materials

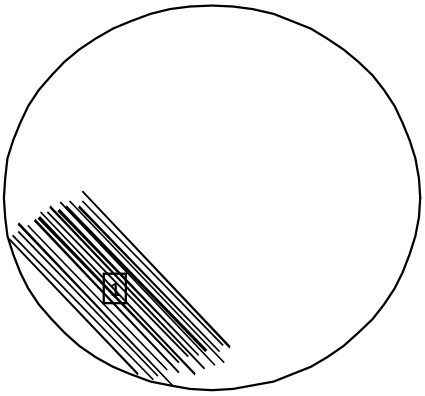
- Bunsen burner
- inoculating loop
- marking pen
- broth culture of microorganism to be streaked
- sterile petri plate of nutrient agar

Procedure

1. Light the Bunsen burner.
2. Place the culture you wish to transfer near the sterile petri plate of nutrient agar.
3. Label the bottom of the petri plate with the name of the microorganism and the date.
4. Hold the inoculating loop with your thumb and first two fingers. Heat the inoculating loop in the Bunsen burner until it is red hot. Heat several inches of the loop, since that much of it will contact the inside of the culture tube. Allow the loop to cool for a few seconds while you hold it in your hand. Do not put it down or allow the loop to touch any surface after it is sterile.
5. While continuing to hold the inoculating loop with your thumb and first two fingers, pick up the tube of culture in your left hand and remove the cap with the last two fingers of your right hand. Keep the cap in your right hand and do not allow it to touch any surface.
6. Using your left hand, draw the open top of culture tube gently through the flame of the Bunsen burner. Do not hold it in the flame for more than a second.
7. Place the sterile loop into the culture tube until the loop is in the liquid. Carefully draw the loop out of the tube and continue to hold the loop in your hand.
8. Draw the open top of the tube gently through the flame of the Bunsen burner and then replace the cap on the culture tub. Replace the tube in its rack.
9. While still holding the loop with the inoculum in your right hand, lift the lid of the petri plate so that it forms about a 45° angle, but is still covering the agar.
10. Insert the loop under the lid of the plate and gently touch the loop to the surface of one edge of the plate, transferring the droplet of culture to the agar.
11. Draw the loop back and forth over the surface of the plate, spreading the droplet over the surface and spreading the bacteria on the plate. The preferred method is called quadrant streaking which is shown in the diagram on the next page. If you use the quadrant streak method, you will flame the loop after you streak each of the 4 quadrants. This gives well-isolated colonies.
12. When the plate is completely streaked, remove the loop and lower the lid.
13. Heat the inoculating loop in the Bunsen burner until it is red hot. You can now place it on the bench or in a rack.
14. At no time should the inoculating loop, the top of the tube, the inside of the cap or the surface of the petri plate have touched any non-sterile surface (especially your fingers or hand).

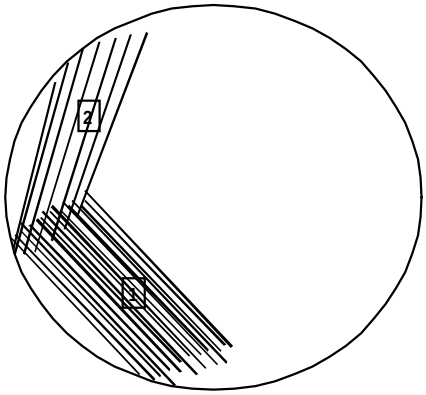
Inoculating a Streak Plate
Quadrant Streak Method

Step 1



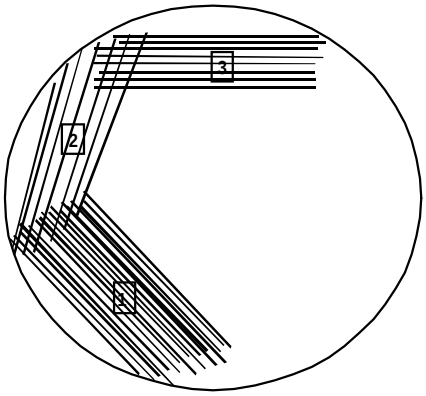
1. Using a sterile inoculating loop, transfer a loopful of broth culture or a single colony from a plate to one edge of the agar plate.
2. Draw the loop back and forth gently over the surface of the agar, spreading the cells over part of the plate as shown above.
3. Flame the loop and cool it by touching an edge of the plate that has not been inoculated.

Step 2



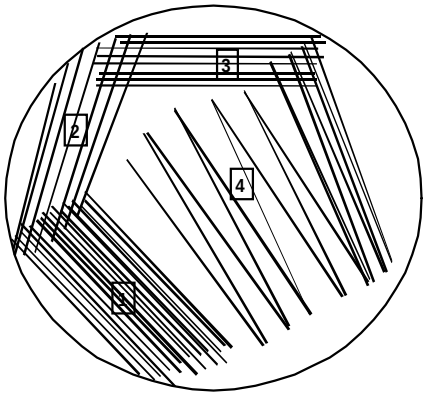
1. Using the cooled sterile inoculating loop, streak from area 1 into area 2 by making a series of straight lines away from the edge of the inoculated area as shown above.
2. Be sure to touch the previously inoculated area 1, but only the edges of it.
3. Make the streaks gently; do not gouge the agar.
4. Flame the loop and cool it by touching an edge of the plate that has not been inoculated.

Step 3



1. Using the cooled sterile inoculating loop, streak from area 2 into area 3 by making a series of straight lines away from the edge of the inoculated area as shown above.
2. Be sure to touch the previously inoculated area 2, but only the edges of it.
3. Make the streaks gently; do not gouge the agar.
4. Flame the loop and cool it by touching an edge of the plate that has not been inoculated.

Step 4



1. Using the cooled sterile inoculating loop, streak from area 3 into area 4 by making a series of zig-zag lines away from the edge of the inoculated area as shown above.
2. Be sure to touch the previously inoculated area 1, but only the edges of it.
3. Make the streaks gently; do not gouge the agar.
4. This area of the plate should have very few bacterial cells, which should produce well isolate colonies.

Results after colonies have grown:
Area 1 - Dense growth
Area 2 - Lighter growth
Area 3 - Sparse growth
Area 4 - Single colonies

Streaking from One Petri Plate to a Fresh Petri Plate

Materials

- Bunsen burner
- inoculating loop
- marking pen
- petri plate containing the microorganism to be streaked (plate A)
- sterile petri plate of nutrient agar (plate B)

Procedure

1. Light the Bunsen burner.
2. Place the petri plate you wish to transfer (plate A) near the sterile petri plate of nutrient agar (plate B).
3. Label the bottom of the petri plate with the name of the microorganism and the date.
4. Heat the inoculating loop in the Bunsen burner until it is red hot. Heat several inches of the loop, since that much of it will contact the inside of the petri plate. Allow the loop to cool for a few seconds while you hold it in your hand. Do not put it down or allow the loop to touch any surface after it is sterile.
5. Lift the lid of petri plate A so that it forms about a 45° angle, but is still covering the agar.
6. Place the sterile loop inside the culture plate A and gently touch the loop to a single bacterial colony. There should not be a large amount of material on the loop.
7. Carefully draw the loop out and replace the lid on the petri plate.
8. While holding the loop with the inoculum in your right hand, lift the lid of the fresh petri plate B so that it forms about a 45° angle, but is still covering the agar.
9. Insert the loop under the lid of the plate and gently touch the loop to the surface of one edge of the plate, transferring the culture material to the agar.
15. Draw the loop back and forth over the surface of the plate, spreading the bacterial cells over the surface of the plate. The preferred method is called quadrant streaking which is shown in the diagram on the previous page. If you use the quadrant streak method, you will flame the loop after you streak each of the 4 quadrants. This gives well-isolated colonies.
10. When the plate is completely streaked, remove the loop and lower the lid.
11. Heat the inoculating loop in the Bunsen burner until it is red hot. You can now place it on the bench or in a rack.

Variations on Transferring Techniques
A sterile swab, pipette or toothpick may be used instead of an inoculating loop. The general rules are the same: allow only a sterile surface to touch the culture and then carefully transfer the culture material to the fresh broth or plate without allowing it to touch any surface. Be particularly careful not to touch any sterile surface with your hands.

Common Causes of Contamination
- Putting a sterile implement on the lab bench after it is sterilized and then picking it up and using it.
- Allowing sterile implements to brush against clothing, arms, hair or hands and then using them.
- Touching the inside of a cap, the top of a sterile test tube or the surface of a plate with hands.
- Placing a contaminated pipette, inoculating loop, toothpick or swab into sterile medium. The medium is no longer sterile, but it still looks sterile for a day or two until the contaminant grows.
- Leaving tubes, flasks, or petri plates open to the air for long period of time. Unless the air is heavily contaminated with microorganisms, it generally requires several minutes of exposure to air to cause contamination.

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