Bacterial Culture Techniques

Culture Media Preparation

Before one can construct a medium that will achieve a desired result in the growth of organisms, one must understand their basic needs. Any medium
that is to be suitable for a specific group of organisms must take into account the following seven factors: water, carbon, energy, nitrogen, minerals, growth factors, and pH. The role of each one of these factors follows

Sterilization

As soon as the tubes of media have been stoppered they must be sterilized. Organisms on the walls of thetubes, in the distilled water, and in the dehydrated medium will begin to grow within a short period of time at room temperature, destroying the medium. Sterilization must be done in an autoclave.

Pure Culture Techniques

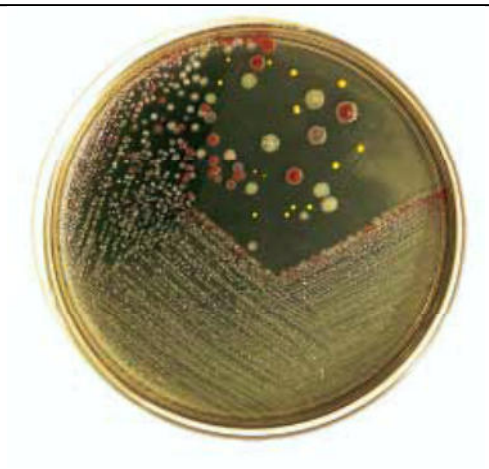
When we try to study the bacterial flora of the body, soil, water, food, or any other part of our environment, we soon discover that bacteria exist in mixed populations. It is only in very rare situations that they occur as a single species. To be able to study the cultural, morphological, and physiological characteristics of an individual species, it is essential,
first of all, that the organism be separated from the other species that are normally found in its habitat; in other words, we must have a **pure culture** of the microorganism. Several different methods of getting a pure culture from a mixed culture are available to us. The two most frequently used methods involve making a streak plate or a pour plate. Both plate techniques involve thinning the organisms so that the individual species can be selected from the others. In this exercise you will have an opportunity to use both methods in an attempt to separate three distinct species from a tube that contains a mixture. The principal difference between the three organisms will be their colors: *Serratia marcescens* is red, *Micrococcus luteus* is yellow, and *Escherichia coli* is white. If

Chromobacterium violaceum is used in place of *M. luteus,* the three colors will be red, white, and purple.

Streak Plate Method

For economy of materials and time, this method is best. It requires a certain amount of skill, however, which is forthcoming with experience. Aproperly executed streak plate will give as good an isolation as is desired for most work. Figure 21.1 illustrates how colonies of a mixed culture should be spread out on aproperly made streak plate. The important thing is to produce good spacing between colonies.

1. Prepare your tabletop by disinfecting its surface with the disinfectant that is available in the laboratory (Roccal, Zephiran, Betadine, etc.). Use a sponge to scrub it clean.
2. Label the bottom surface of a sterile Petri plate with your name and date. Use a china marking pencil.
3. Liquefy a tube of nutrient agar, cool to 50° C, and pour the medium into the bottom of the plate, following the procedure illustrated in figure 21.2. Be
sure to flame the neck of the tube prior to pouring to destroy any bacteria around the end of the tube. After pouring the medium into the plate, gently
rotate the plate so that it becomes evenly distributed, but do not splash any medium up over the sides.
4. Streak the plate by one of the methods shown in figure 21.4. Your instructor will indicate which technique you should use. **Caution:** Be sure to follow the routine in figure 21.3 for getting the organism out of culture.
5. Incubate the plate in an *inverted position* at 25° C for 24–48 hours. By incubating plates upside down, the problem of moisture on the cover is minimized.



POUR PLATE METHOD
(Loop Dilution)

This method of separating one species of bacteria from another consists of diluting out one loopful of organisms with three tubes of liquefied nutrient agar in such a manner that one of the plates poured will have an optimum number of organisms to provide good isolation. Figure 21.5 illustrates the general procedure. One advantage of this method is that it requires somewhat less skill than that required for a good streak plate; a disadvantage, however, is that it requires more media, tubes, and plates. Proceed as follows to make three dilution pour plates, using the same mixed culture you used for your streak plate.

1. Label the three nutrient agar pours **I, II,** and **III** with a marking pencil and place them in a beaker of water on an electric hot plate to be liquefied. To save time, start with hot tap water if it is available.
2. While the tubes of media are being heated, label the bottoms of the three Petri plates **I, II,** and **III.**
3. Cool down the tubes of media to 50° C, using the same method that was used for the streak plate.
4. Following the routine in figure 21.5, inoculate tube I with one loopful of organisms from the mixed culture. Note the sequence and manner of handling the tubes in figure 21.6.
5. Inoculate tube II with one loopful from tube I after thoroughly mixing the organisms in tube I by shaking the tube from side to side or by rolling the tube vigorously between the palms of both hands. ***Do not splash any of the medium up onto the tube closure.*** Return tube I to the water bath.
6. Agitate tube II to completely disperse the organisms and inoculate tube III with one loopful from tube II. Return tube II to the water bath.

7. Agitate tube III, flame its neck, and pour its contents into plate III.
8. Flame the necks of tubes I and II and pour their contents into their respective plates.
9. After the medium has completely solidified, incubate the *inverted* plates at 25° C for 24–48 hours.

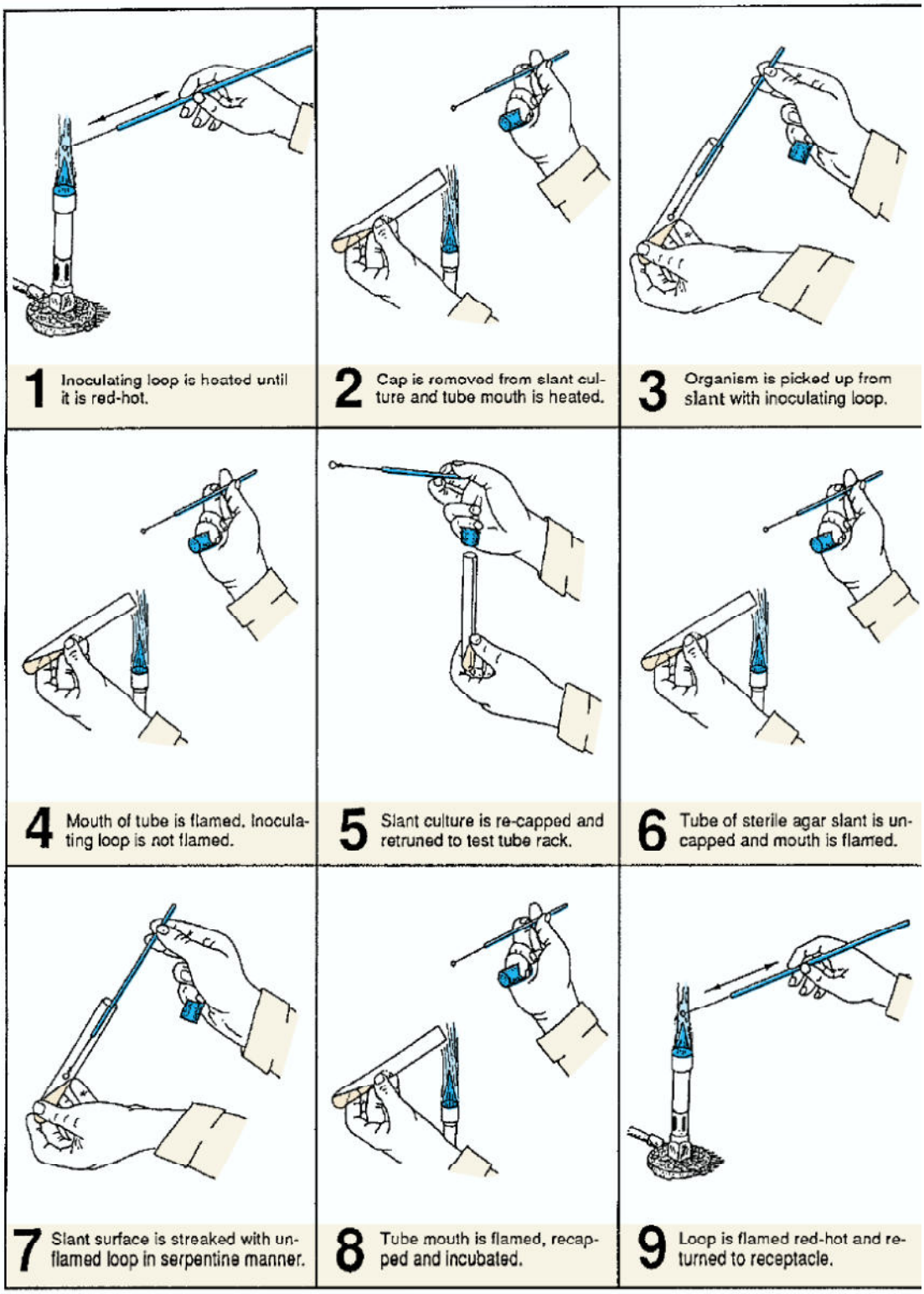
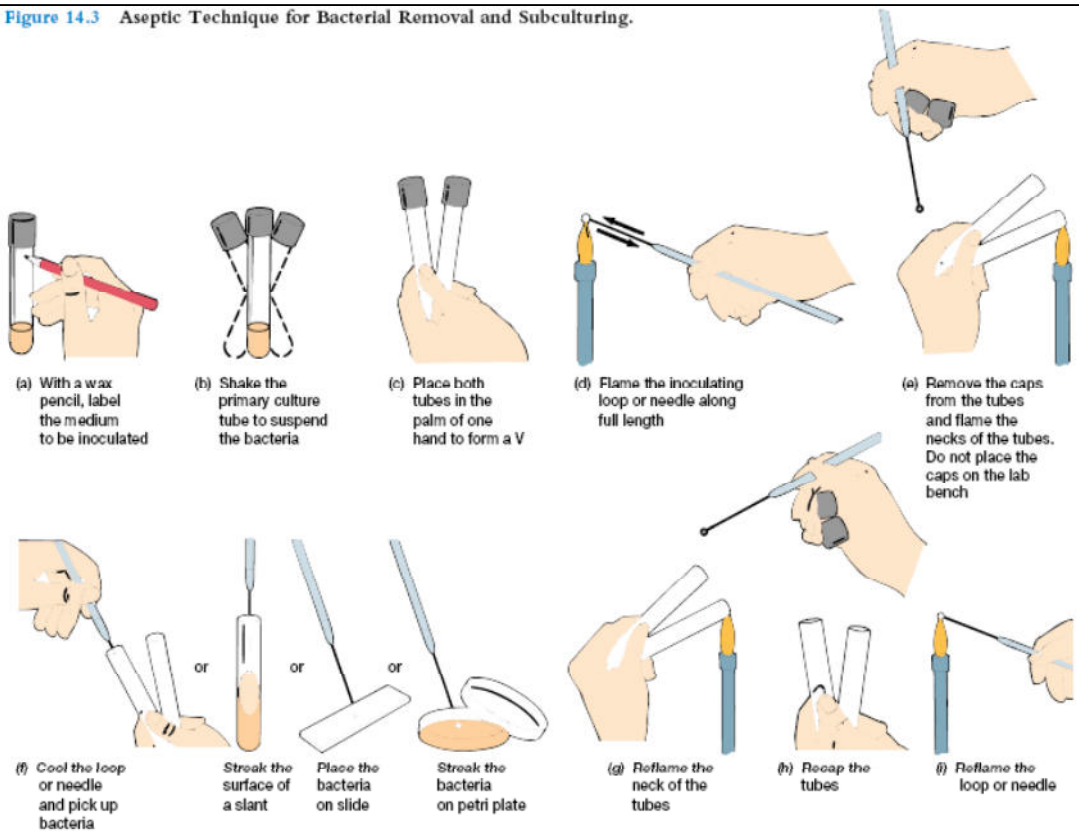


Figure 8.4 Procedure for inoculating a nutrient agar slant from a slant culture

Figure 14.3 Aseptic Technique for Bacterial Removal and Subculturing.



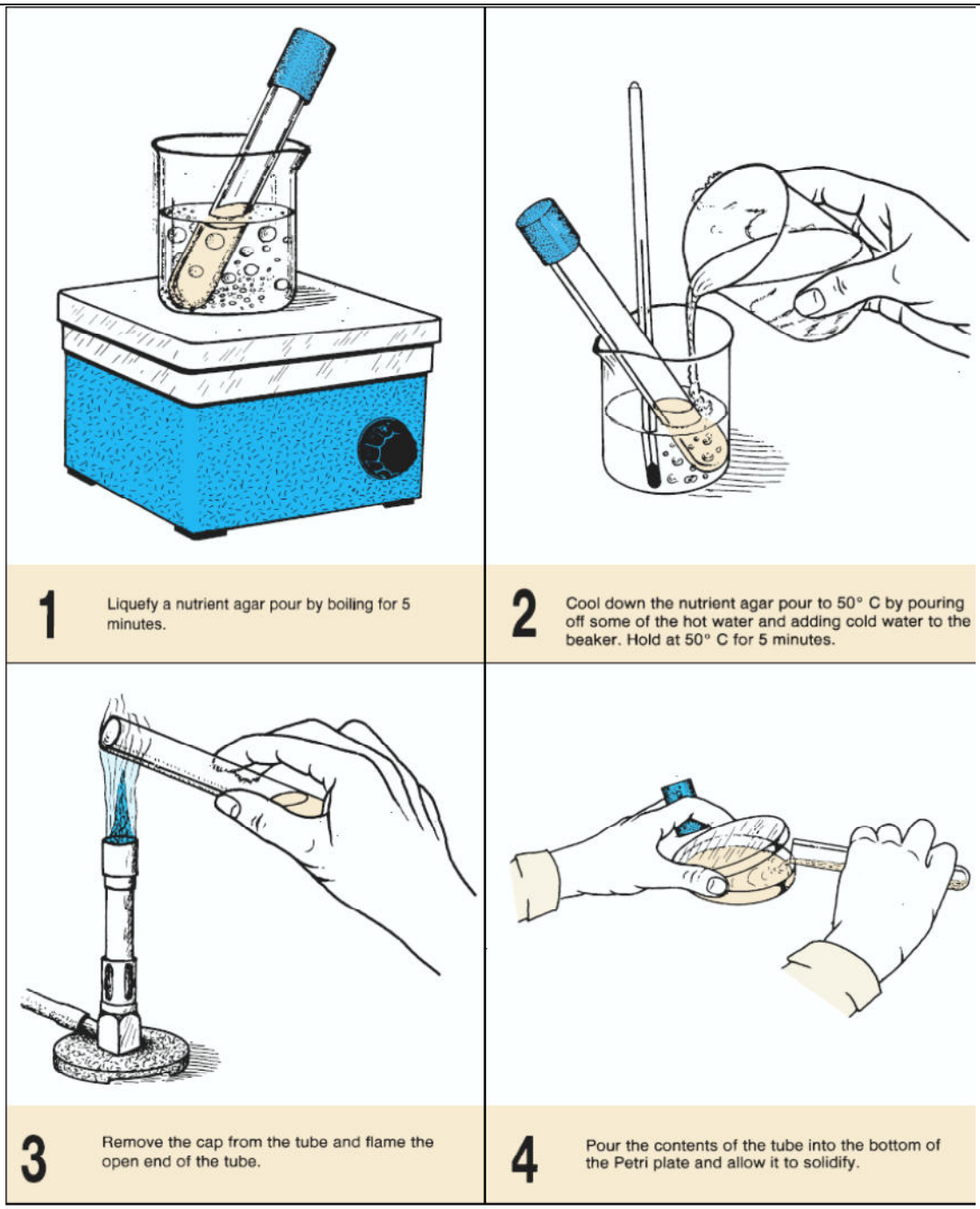


Figure 21.2 Procedure for pouring an agar plate for streaking

Exercise 21 • Pure Culture Techniques

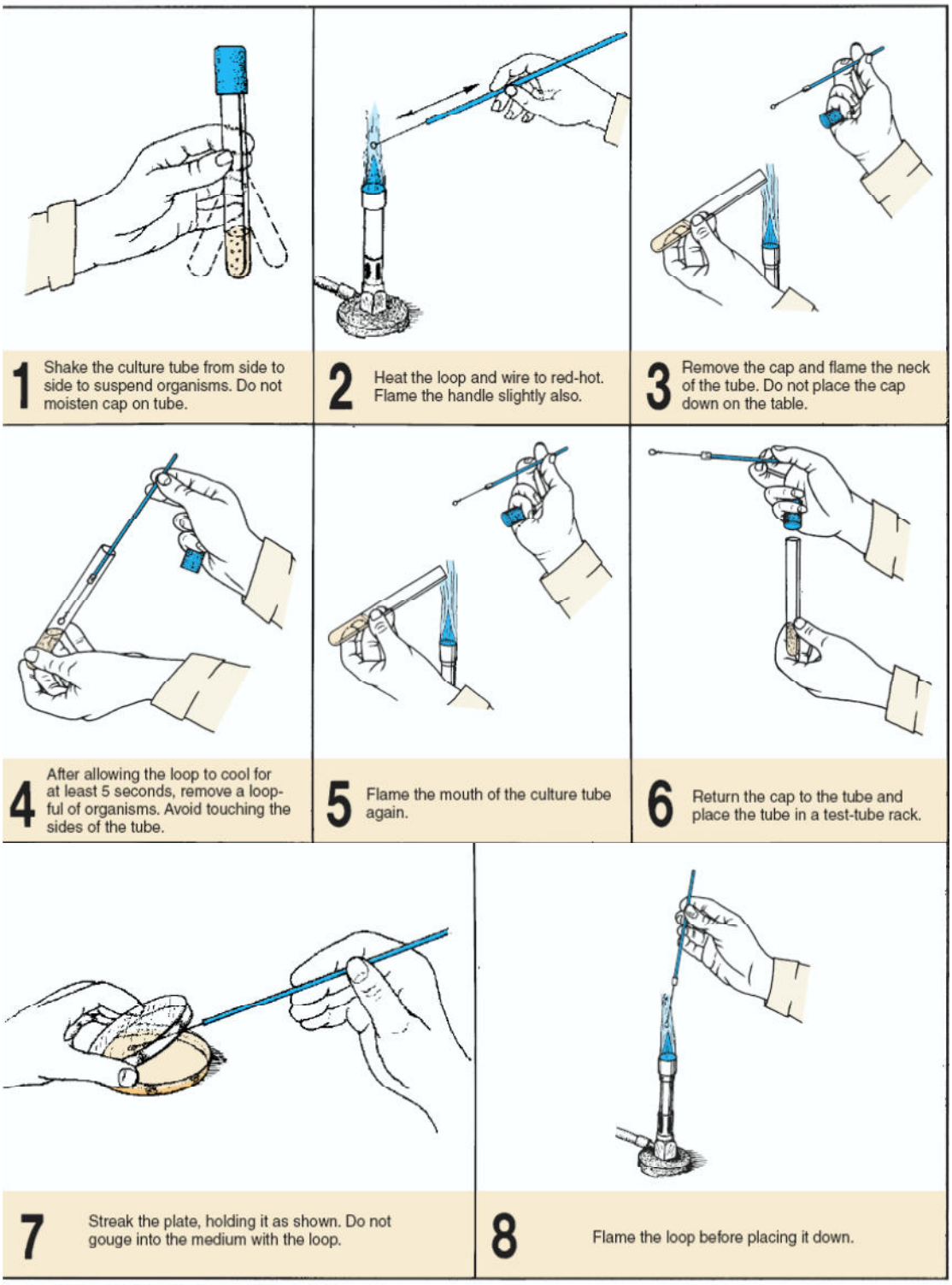
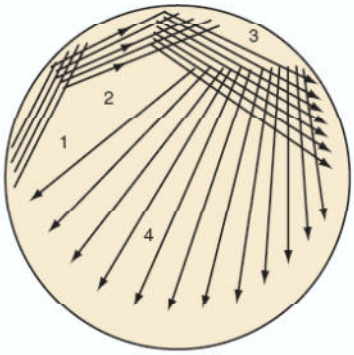
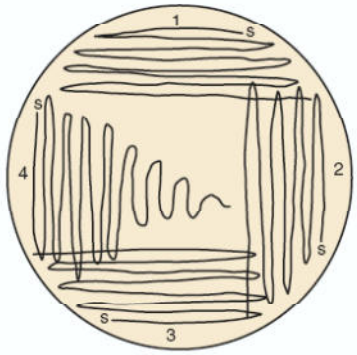


Figure 21.3 Routine for inoculating a Petri plate



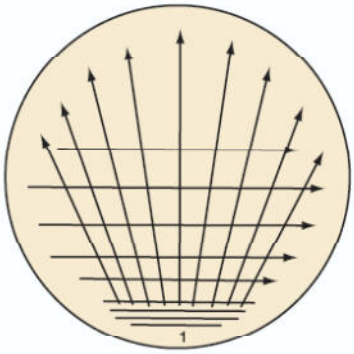
QUADRANT STREAK
(Method A)

1. Streak one loopful of organisms over Area 1 near edge of the plate. Apply the loop lightly. Don't gouge into the medium.
2. Flame the loop, cool 5 seconds, and make 5 of 6 streaks from Area 1 through Area 2. Momentarily touching the loop to a sterile area of the medium before streaking insures a cool loop.
3. Flame the loop again, cool it, and make 6 or 7 streaks from Area 2 through Area 3.
4. Flame the loop again and make as many streaks as possible from Area 3 into Area 4, using up the remainder of the plate surface.
5. Flame the loop before putting it aside.



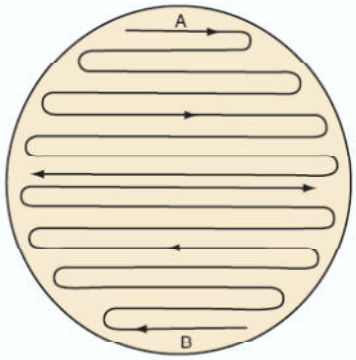
QUADRANT STREAK
(Method B)

1. Streak one loopful of organisms back and forth over Area 1, starting at point designated by "s". Apply loop lightly. Don't gouge into the medium.
2. Flame the loop, cool 5 seconds and touch the medium in sterile area momentarily to insure coolness.
3. Rotate the dish 90 degrees while keeping the dish closed. Streak Area 2 with several back and forth strokes, hitting the original streak a few times.
4. Flame the loop again. Rotate the dish and streak Area 3 several times, hitting last area several times.
5. Flame the loop, cool it, and rotate the dish 90 degrees again. Streak Area 4, contacting Area 3 several times and drag out the culture as illustrated.
6. Flame the loop before putting it aside.



RADIANT STREAK

1. Spread a loopful of organisms in small area near the edge of the plate in Area 1. Don't gouge medium.
2. Flame the loop and allow it to cool for 5 seconds. Touching a sterile area of the medium will insure coolness.
3. **From the edge** of Area 1 make 7 or 8 straight streaks to the opposite side of the plate.
4. Flame the loop again, cool it sufficiently, and cross streak over the last streaks, **starting near Area 1.**
5. Flame the loop again before putting it aside.



CONTINUOUS STREAK

1. Starting at the edge of the plate (Area A) with a loopful of organisms, spread the organisms in a single continuous movement to the center of the plate. Use light pressure and avoid gouging the medium.
2. Rotate the plate 180 degrees so that the uninoculated portion of the plate is away from you.
3. Without flaming loop, and using the same face of the loop, continue streaking the other half of the plate by starting at Area B and working toward the center.
4. Flame your loop before putting it aside.

Figure 21.4 Four different streak techniques

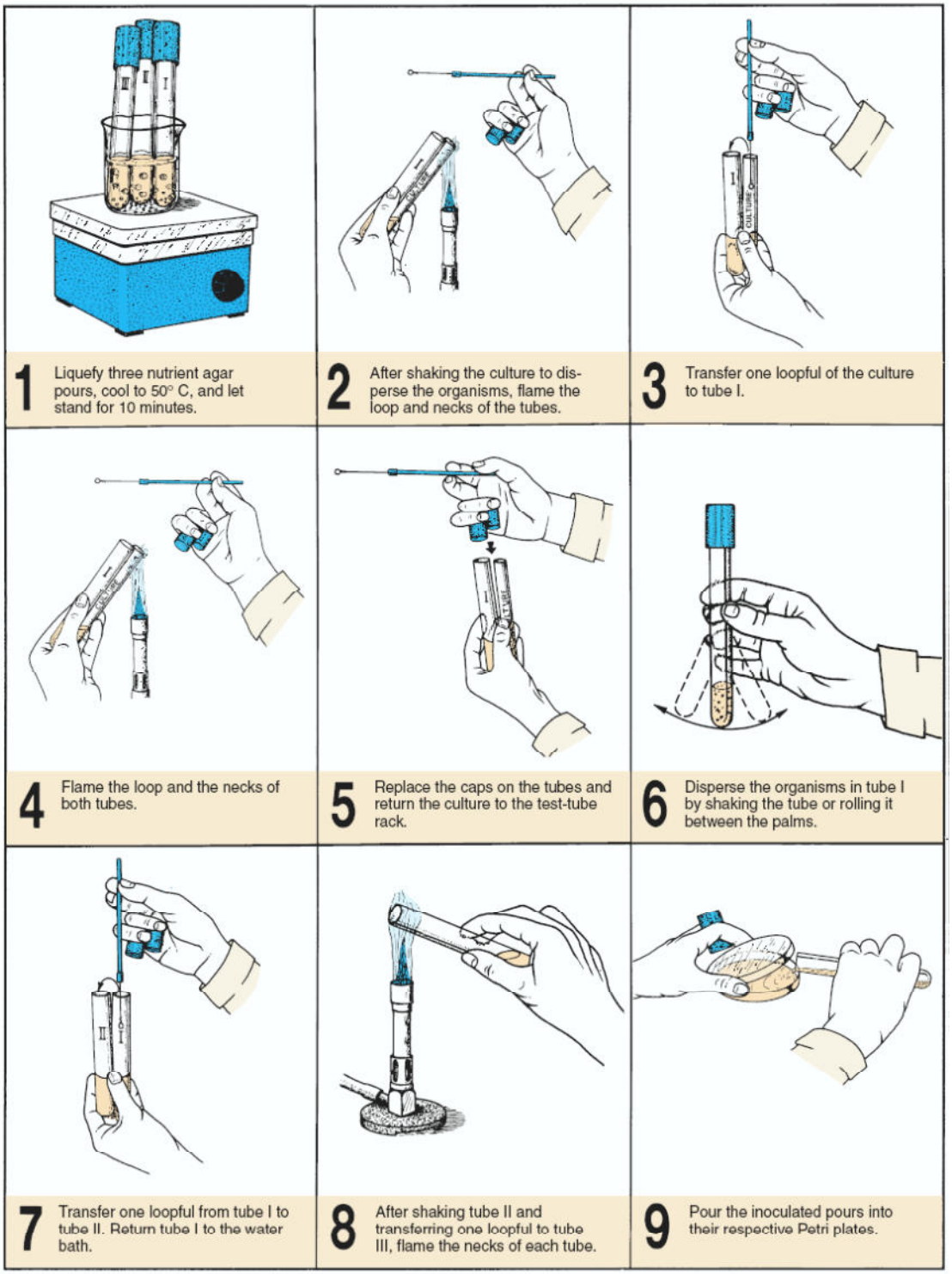


Figure 21.6 Tube-handling procedure in making inoculations for pour plates

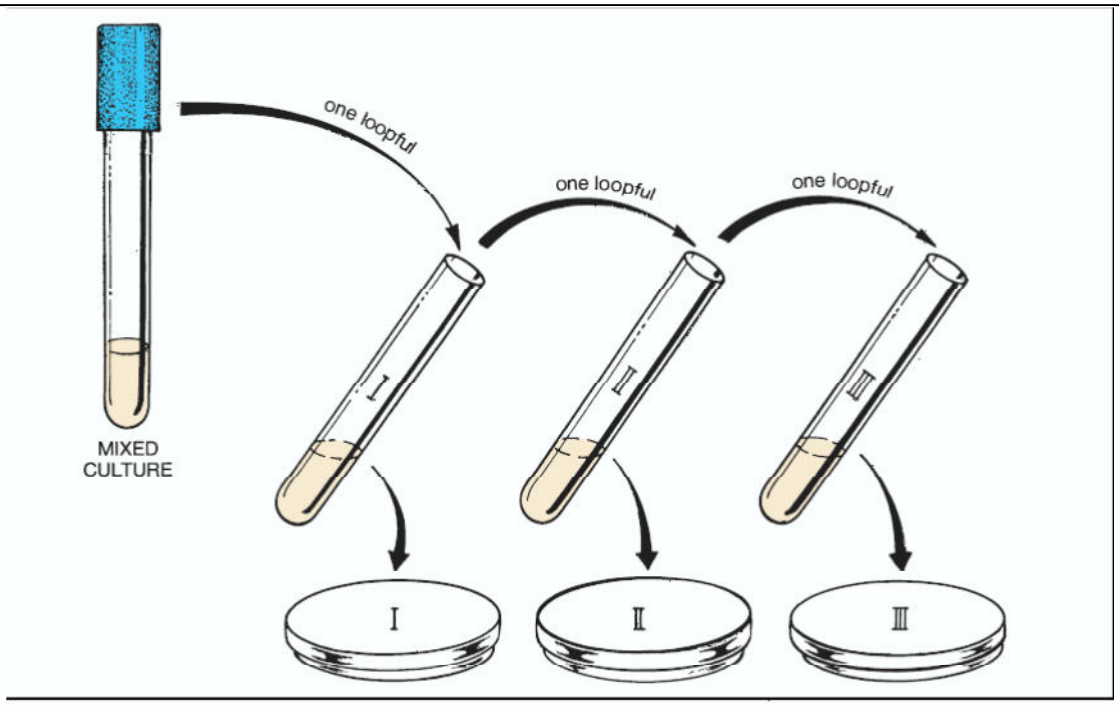


Figure 21.5 Three steps in the loop dilution technique for separating out organisms