Procedure for Counting Bacterial Cells via Serial Dilution

Background: Bacterial cells are very small and can be difficult to count from a slide under a microscope. They also grow to a high density in a liquid culture, which can also make counting them rather tricky. When a bacterial culture in liquid media is introduced to an agar plate, each individual viable bacterium will form a colony, which will be visible to the eye (assuming the agar provided the nutrients this bacterium needs). The colonies can then be counted and you will then be able to extrapolate the number of viable bacteria in the entire culture.

Materials:

3 agar plates	3 sterile swabs	1 sterile transfer pipette
20 ml sterile media	2 sterile tubes	

1. Label 3 agar plates on the bottom: 1. Direct Count, 2. 1/10 Dilution, 3. 1/100 dilution. Label all plates with your initials, class, and date.
2. Choose one of your cultures to test, close the lid, and invert it 3 times so it is mixed well.
3. Dip a sterile swab into your culture, press it against the side of the tube to squeeze out excess liquid, gently rub the wet swab over the surface of the agar plate labeled “direct count.”
4. Label the two tubes 1/10 and 1/100.
5. Using the transfer pipette, fill each of the 2 empty sterile tubes with 9 mL of sterile media. Be careful not to touch the sides of the tubes or the table with the pipette.
6. Withdraw 1 mL of your culture. Add to the tube labeled 1/10. Mix well by swishing the solution in and out of the pipette. Withdraw 1mL of this solution. Add to the tube labeled 1/100.
7. Dip a sterile swab into the tube labeled 1/10, press it against the side of the tube to squeeze out excess liquid, gently rub the wet swab over the surface of the agar plate labeled 1/10 dilution.
8. Dip a sterile swab into the tube labeled 1/100, press it against the side of the tube to squeeze out excess liquid, gently rub the swab over the surface of the agar plate labeled 1/100 dilution.
9. Discard the tubes containing your dilutions according to your teacher’s instructions.
10. Incubate the plates for 48 hours, then count and record the number of colonies on each plate. If there are more than 200 colonies, record the data as TNTC (too numerous to count).
11. Calculate the number of bacteria from the swab of your culture using the formula:

Number of bacteria = number of colonies/dilution factor