Exercise 13
DETERMINATION OF MICROBIAL NUMBERS

Introduction

When biologists discuss the growth of microorganisms (**microbial growth**), they are actually referring to **population size** rather than to the size of individual cells. Although individual bacteria cells do elongate during the fission process, the extent of this "growth" is minimal when compared to that of multicellular organisms. The growth of a bacterial population (in vitro or in their natural environment), is much more extensive. A typical **colony** (as visible on agar plates) contains hundreds of thousands of cells, and a broth culture may contain billions. Since bacteria reproduce exponentially, their populations can achieve these large numbers very quickly. For this reason, it was essential that microbiologists develop methods to determine population size. Some of these methods involve the determination of cell numbers while others measure the overall mass of a population in grams. In either case, determinations of microbial growth usually involve taking very small samples and from those calculating the size of the total population.

One of the most common methods used for measuring bacterial cell numbers (i.e., the number of cells in a given batch of media) is the standard plate count or colony count. This method is based on the premise that each bacterial colony observed growing on a plate originated from a single cell. By counting the number of colonies present on a plate, and knowing the **dilution factor** (the extent to which the population density has been decreased), one can determine the number of viable cells present in the original culture. One advantage of the standard plate count over some other methods of determining microbial numbers is that it reflects only the number of **viable** (living) cells present.

Other methods used to determine bacterial numbers include dilution counts using pour plates, membrane filter counts, direct microscopic counts, electronic device (Coulter counter) counts, turbidimetric methods, chemical estimates, and dry weight or cell volume measurements.

Quantitative Plating Method or Viable Cell Count

To obtain a bacterial count using the plate method, a bacterial culture is diluted stepwise through a series of tubes or bottles containing known amounts of solution; this process is called **serial dilution**. The number of cells present in the original sample is then determined by multiplying the number of colonies that develop by the degree of dilution (total dilution factor) of the plate being counted. Dilutions are usually expressed as negative exponents rather than as fractions (i.e., 10^{-5} rather than 1/100,000). The population number is usually recorded as the number of cells or **colony forming units** (cfu) per milliliter of liquid being examined (water, milk, broth media, etc.).

Procedure:

1. Obtain a broth culture of the bacteria provided and swirl it gently to be sure the cells are evenly distributed.

2. Obtain three bottles of sterile water, each of which contains approximately 99 mL, and label them 10^{-3}, 10^{-5}, and 10^{-7}.

3. Obtain 3 agar plates (nutrient or TSA as indicated) and label them 10^{-5}, 10^{-6}, and 10^{-7}.

4. Using a sterile pipette, stir your bacterial culture to mix it thoroughly, and then transfer 0.1 mL of culture media to the water blank labeled 10^{-3}.

   Note - If you wish to obtain maximal accuracy, you may also add 0.9 mL of sterile water to the water blank, bringing its volume to exactly 100 mL.

5. Close the inoculated water blank and shake it vigorously (about 25 times) to thoroughly mix the sample and to break up any clumps of bacteria.

6. Using a second sterile pipette, transfer 1.0 mL of the solution from the sample labeled 10^{-3} into the water blank labeled 10^{-5}.

7. Mix this sample thoroughly as in step #5 above.

8. Using a third sterile pipette, transfer 1.0 mL of the solution from the sample labeled 10^{-5} into the water blank labeled 10^{-7}. (**Note** - If you carefully slip the pipette used for this step into its sterile plastic cover, you will be able to use it again in step 10.)

9. Mix this sample thoroughly as in step #5 above.

10. Using a fourth sterile pipette (or the pipette retained from step 8), transfer 1.0 mL of the solution labeled 10^{-5} onto the agar plate labeled 10^{-5} and then transfer 0.1 mL from the same water bottle onto the plate labeled 10^{-6}.

11. Using a fifth sterile pipette, transfer 1.0 mL of the solution labeled 10^{-7} onto the agar plate labeled 10^{-7}.

12. Starting with the most dilute sample (10^{-7}) and working backward, spread the inoculum evenly over the entire surface of each plate using a sterile glass spreader as demonstrated. If handled properly, the same spreader can be used on all three plates without sterilizing it between them.

13. There will be some excess water on the agar surface of some of your plates. Tape the plates together in a stack of three, and then invert them quickly so the water is not allowed to run to one side. Incubate all plates at 37^{o} C until the next laboratory period.

14. Count the colonies present on each plate containing between 20 and 200 colonies.

15. Calculate the population density (i.e. the number of bacteria contained in 1.0 mL of the original broth culture) by multiplying the number of colonies counted times the dilution factor expressed as a **positive exponent**.

    Example:
    If you count 50 colonies on the plate labeled 10^{-7}, you will multiply 50 times 10^{7}, so the number of viable cells or colony-forming units (cfu) in the original culture was 5×10^{8} cfu/ml. (**Note** - When numbers are expressed in scientific notation there may be only one digit to the left of the decimal point. As the decimal point is moved one place to the left, the exponent is increased by one.) This means the original sample contained 500,000,000 cells or cfu per mL.

Name _____ **Lab Section** _____

WORKSHEET
Exercise 13
Determination of Microbial Numbers

Goals: _____
_____
_____

Materials & Methods:
Viable Cell Count
Date: _____ Organism type used: _____
Medium used: _____
Incubation temperature: _____ Duration of incubation: _____

Draw a diagram and fill in the table to represent the serial dilution you performed.

Individual Dilution Factor	
Total Dilution Factor	

Data & Results:

Plate Number			
Number of Colonies			

Conclusions:

How many viable bacterial cells were present in 1 mL of your original bacterial culture? _____
_____

How many viable bacterial cells were present in 5 mL of your original bacterial culture? _____
_____

You should have observed the greatest number of colonies on the first plate and the least number of colonies on the last plate. Did you? _____ What do your observations of colony number tell you about the success of your dilution technique? _____
_____
_____
_____
_____

Questions:

1. Why would a scientist/technician want to know how many viable bacterial cells are present in a sample? Give one example of a situation in which this information would be important to know.

2. If the initial inoculum used at step 4 above was 1.0 mL instead of 0.1 mL, what degree of dilution would be reached in the third water blank?

3. Approximately how many viable bacterial cells or colony forming units were present in 1.0 mL of your original bacterial culture?

4. Do all bacterial cultures (in the stationary phase of growth) contain similar numbers of cells? What explanation might be given for the variation in population observed?