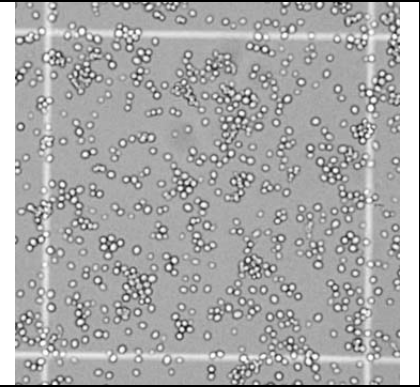


Lab Six
Enumerating Microbes

Viable Counting
Microscopes and Counting Chambers
Spectrophotometer-Based Counting

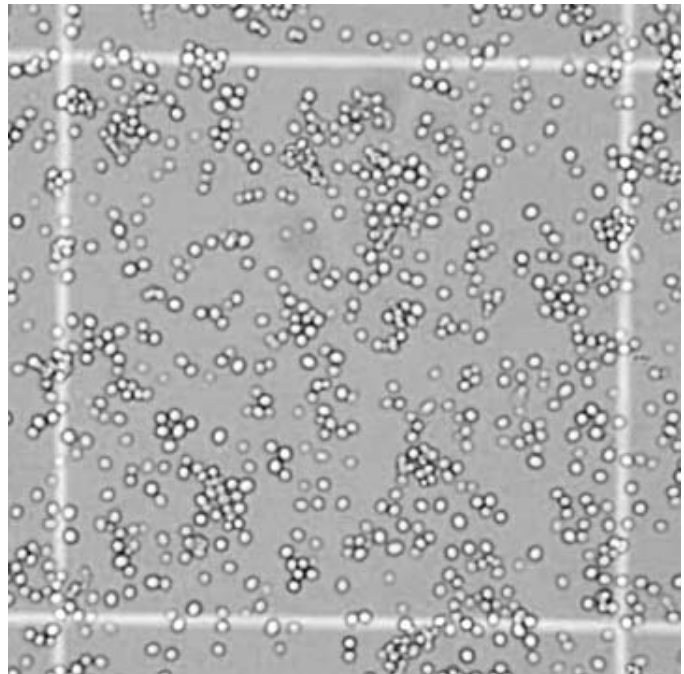
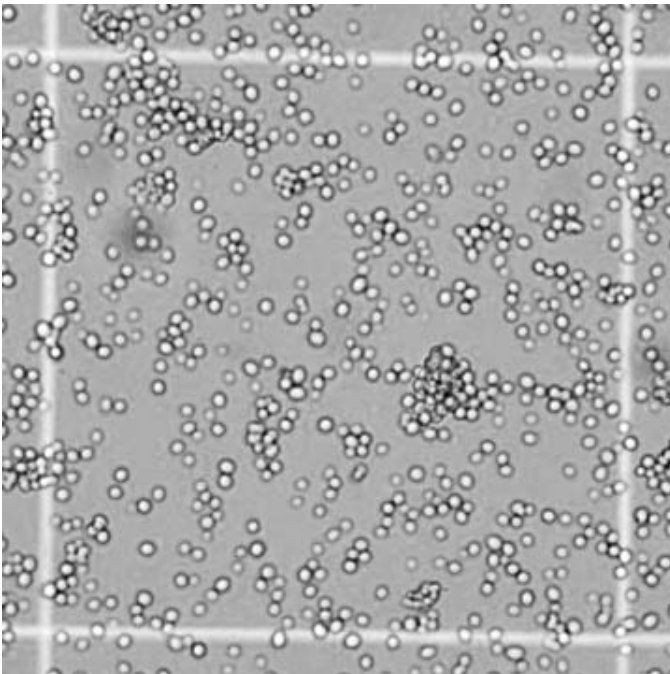


Part One - Viable Counting Using Plates

Review Ch. 8 sections about viable counting. Although useful for counting live cells, many microbes cannot be grown using lab media, and growing overnight is not rapid. In this experiment, you will be given a tube with MANY Serratia. Your job is to figure out how many cells/ml there are. Because plating directly would yield a lawn, you first need to make a dilution series. For plating, you will use a dally rod that cannot be heated to red-hot and cooled. Instead, you will sterilize the rod by dipping it in ethanol and burning off the residual liquid.

Procedures

Obtain 6 test tubes, each with 9 ml water; Label 1-6; 1 will be the least dilute and 6 the most dilute
Using a sterile transfer pipette, remove 1 ml Serratia, add to test tube 1, and mix
Using a new pipette, remove 1 ml from tube 1, add to tube 2, and mix - repeat down the line to tube 6
Now obtain 6 nutrient plates and label with your name and #1-6, reflecting the dilution series
Using a sterile transfer pipette, transfer 0.1 ml liquid from tube 1 to plate 1
Using a alcohol/flame-sterilized dally rod, spread liquid evenly - repeat with all test tubes
Next week, count colonies and calculate how many cells/ml were in the original tube



Part Two - Microscopic Counting - DRY LAB EXERCISE

Review text about microscope-based counting using a cell counting chamber (e.g. a hemacytometer). Although this method is rapid (i.e. no overnight growth), it does not distinguish between living and

dead cells - and can be difficult if cells are clumpy or moving. A hemacytometer allows scientists to place exactly 0.0001 ml over a grid with many squares. Counting ONE SQUARE (i.e. the cells within the white square boundaries above) gives you the number of cells per 0.0001 ml. Determine counts for the 2 grid images provided above - yes, they are different. Use this information to back-calculate how many cells/ml were in original sample.

Part Three - Counting Using a Spectrophotometer - DRY LAB EXERCISE

Review text about spectrophotometer-based counting. Although this method is rapid, it also does not distinguish between living and dead cells. Also, you need to run control standards so that you can graph a standard curve (X = cells/ml and Y = absorbance). By locating unknown absorbances on the graph, you can determine cells/ml. Use the following information to graph a standard curve and determine unknown concentrations.

<u>Sample</u>	<u>Concentration</u>	<u>Absorbance</u>
Standard 1	10,000 cells/ml	20
Standard 2	8,000 cells/ml	16
Standard 3	6,000 cells/ml	12
Standard 4	4000 cells/ml	8
Standard 5	2,000 cells/ml	4
Standard 6	1,000 cells/ml	2
Dirty Sponge Water	Unknown	5
UTI Urine Sample	Unknown	17
Ground Beef Water	Unknown	15

Biology 318 Worksheet Due Next Lab - TURN IN ONE WORKSHEET ONLY PER PAIR

Names:

(1) 6 pts. Viable Count Data. 300 or more = TMTTC (too many to count).

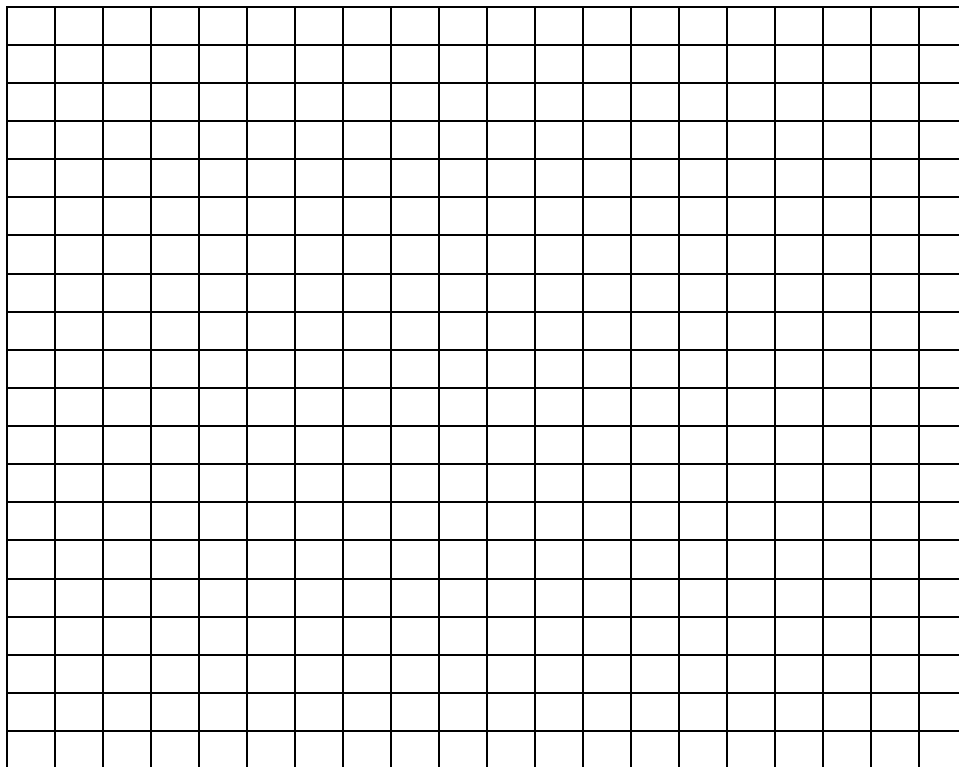
<u>Plate</u>	<u>Observed Number of Colonies</u>
1 (least dilute)	
2	
3	
4	
5	
6 (most dilute)	

(2) 6 pts. Using any count between 30-300 in the above data table, calculate the number of organisms per ml in the original sample. Remember: you only plated 0.1 ml of 10 ml. This HAS TO BE figured into back-calculation. Make a drawing of your entire dilution and plating series and use it to do these calculations. SHOW ALL MATH.

(3) 5 pts. Microscope-Based Counting Table. Below, SHOW ALL MATH for converting from grid counts to cell/ml counts.

Grid 1 (#cells in 0.0001 ml)	
Grid 2 (#cells in 0.0001 ml)	
Average of Both Grids	
Predicted cells per 1 ml	

(4) 8 pts. Spectrophotometer-Based Graph and Calculations. On the graph, trace your standard curve in red; label and circle each unknown along the standard curve.



<u>Unknown</u>	<u>Estimated Concentration</u> (Cells/ml)
Dirty Sponge Water	
UTI Urine Sample	
Ground Beef Water	