

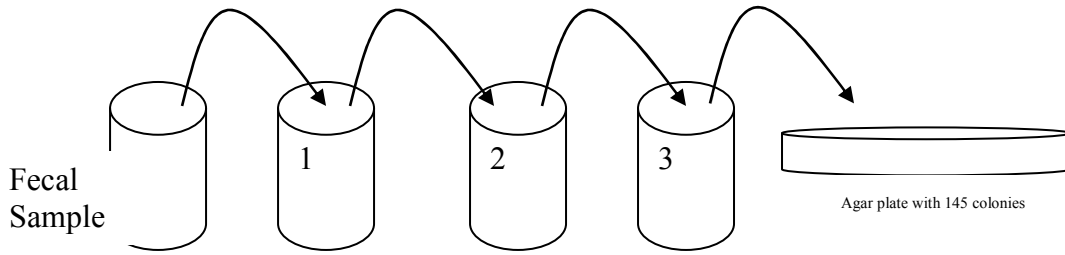
Identification/Applications: For Practicals

1. Know how to count a plate of colonies.
2. Know how to use the spec 20.
3. Be able to convert % transmittance to OD

Concepts: For quizzes and practicals

1. What is the difference between direct and indirect counting methods for bacteria (you may need to use PowerPoint)?
2. What is the difference between viable and total counting methods for bacteria (you may need to use PowerPoint)?
3. What are examples of direct and indirect counting methods and viable and total counting methods? Make sure you know the ones discussed in lab.
4. Explain how the turbidity of a culture relates to its cell density, OD and % transmittance.
 - a. Example: As the turbidity increases the OD _____; the cell density _____; the % transmittance _____.
 - b. Example: As the cell density increases the OD _____; the % transmittance _____.
5. Distinguish between serial dilution and total dilution.
6. What does percent transmittance mean? What does OD mean?
7. When doing the heterotrophic plate count method to determine viable cells in a culture:
 - a. Why must you plate each dilution 3 times?
 - b. Why must you not count the plates that have over 300 colonies or under 30 colonies to determine viable counts?
8. When doing the turbidimetry method to determine cell numbers:
 - a. Why must you use clean cuvettes?
 - b. Why must you agitate the tubes before inserting them in the spectrophotometer?
9. When is it appropriate to use the turbidimetry method to count bacteria and when is it appropriate to perform the heterotrophic plate count method?

10. Be able to perform the following calculations (or ones like them):
If you have a fecal sample and you performed the following dilution series.



You transfer 0.1 ml of sample from to a fresh tube with 9.9 ml of media. Next you transfer 1 ml from tube 1 to 9.0 ml of media in tube 2. Finally, you transfer 1 ml of tube 2 to 9 ml of media in tube 3. Then you transfer 0.1 ml from tube 3 to an agar plate. The plate is incubated for 24 hours then you count 145 colonies on the plate. Answer the following questions.

1. Determine the serial and total dilutions at each tube (1, 2, and 3).
2. Determine the number of cells per ml in tube 3.
3. Determine the number of cells per ml in the fecal sample.

A patient comes in with an obvious urinary tract infection. It appears she is infected with *E. coli*. A urine sample is taken to determine the number of *E. coli*. The sample is diluted down and plated on media that selects for *E. coli*. Note the dilution scheme and answer the questions.

Dilution Scheme: 0.5 ml is taken from the urine sample and transferred to 4.5 ml of sterile media in tube 1 (see below). Then 5 ml of sample is transferred from tube 1 to tube 2, which contains 5 ml of sterile media. Finally 0.1 ml is transferred from tube 2 to an agar plate. If you counted 203 colonies on the plate after 24 hours, determine the number of cells/ml of urine.

Note: It is quite healthy to have E. coli in your intestine, but when E. coli is accidentally transferred to the urinary tract it can grow to large numbers and cause a UTI.

