# INS 099 Data Analysis Summer 2007

Day 1: Solutions, Dilutions, and Exponential Growth **Due Tues. July 17, 2007**

## Microbial population counts

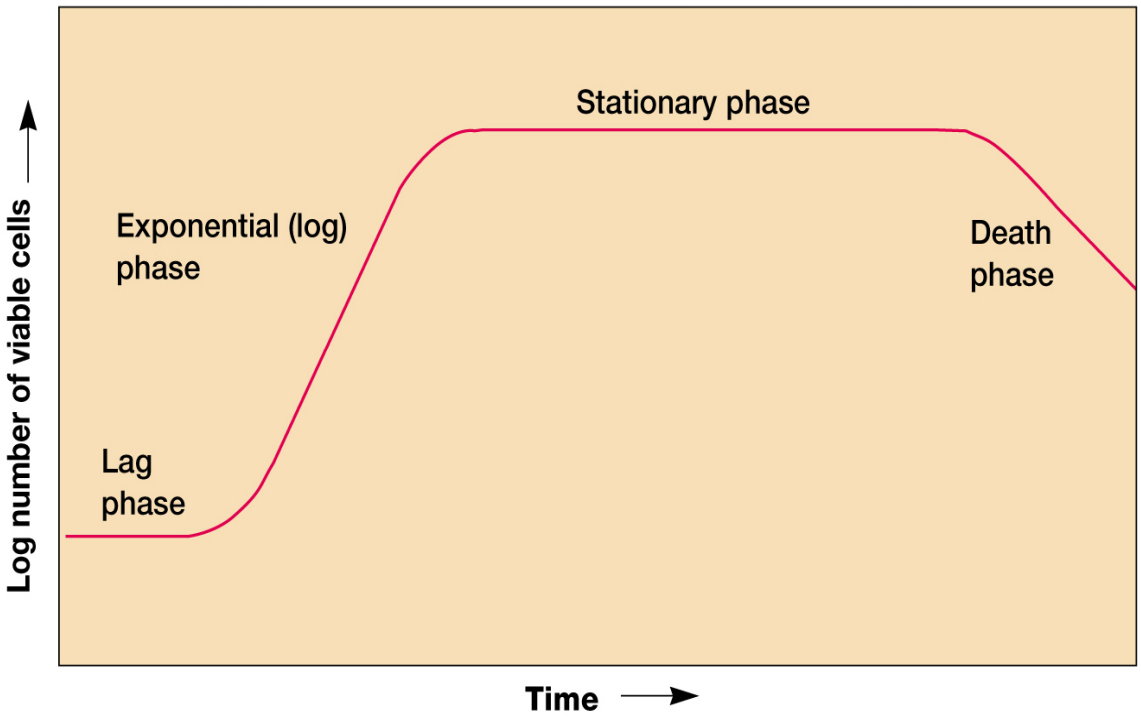
## Exponential Growth

**If $N_0$ is the initial population and the growth rate is *k* then the population *N* at time *t* is: $N_f = N_0 e^{kt}$, where k > 0.**

**Can be simplified to, generation or doubling time: $N_f = N_0 \times 2^n$**

**Example of growth curve:**






**Plot the data in file P:/Temp, Folder "Week 3" labeled "INS_1_Rob". Print your plots and identify each phase of the growth curve.**

1) **What difference do you notice between these two organisms? Explain.**

**2) What might be the reason for this difference?**

## DETERMINING MICROBIAL POPULATIONS:

Microorganisms are often counted in the laboratory using methods such as the viable plate counting where a dilution of a sample is plated on an agar medium. After incubation, plates with 30-300 colonies per plate are counted. This number of colonies (30-300) was chosen because the number counted is high enough to have statistical accuracy, yet low enough to avoid nutrient competition among the developing colonies. Each of the colonies is presumed to have arisen from only one cell, although this may not be true if pairs, chains, or groups of cells are not completely broken apart before plating. It is possible, even though unlikely, for an original (undiluted) sample of microorganisms to have 30-300 cells/ml so that no dilution will be necessary to give good results. More likely, a sample will have greater numbers of cells/ml; sometimes, as in the case of unpolluted water samples, the sample will have less. In either case, the sample must be manipulated so that it contains a number of cells in the correct range for plating. If the cell number is high, the sample is diluted. Dilutions are performed by careful, aseptic pipetting of a known volume of sample into a known volume of a sterile buffer, water, or saline. This is mixed well and can be used for plating and/or further dilutions. If the number of cells/ml is unknown, then a range of dilutions are usually prepared and plated.

In order to make the calculation of the number of cells/ml in the original sample easier, dilutions are designed to be easy to handle mathematically. The most common dilutions are 1/10, 1/100, and 1/1000. Looking first at the 1/10 (or $10^{-1}$) dilution, it can be made by mixing 1 ml of sample with 9 ml of sterile dilution buffer.

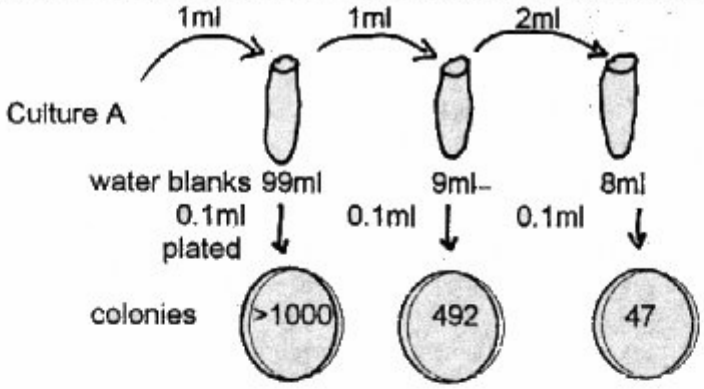
**Problem:** If we have 1 ml of a culture of *Escherichia coli* containing between 30 – 300 cells/ml, how can we determine the precise population count; representing the actual number of cells in the 1 ml (of culture dilution) used?

**How do we need to start? Illustrate by drawing.**

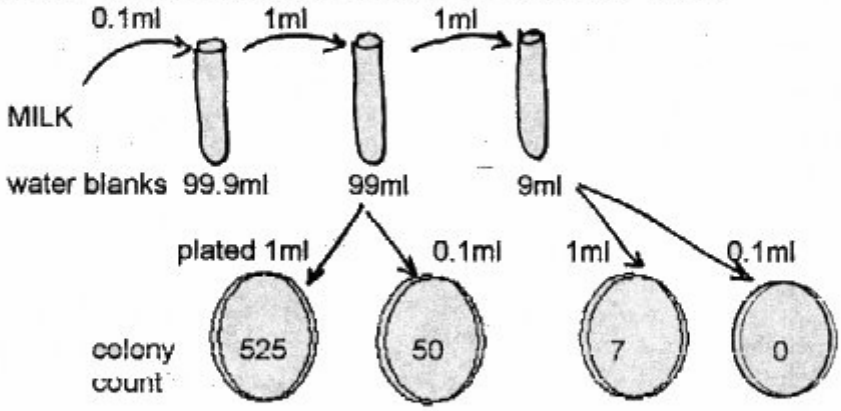
Assumptions: 1) *E. coli* cells don't die when they are mixed into agar at ~50°C.
2) Each cell, if culture is mixed well into agar, will give rise to one colony.

SAMPLE PROBLEMS:

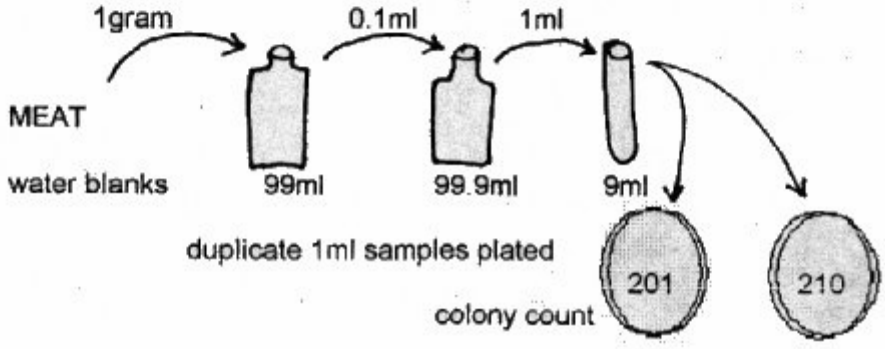
CALCULATE THE NUMBER OF BACTERIA PER MILLILITER OF CULTURE A.




CALCULATE THE NUMBER OF BACTERIA PER ML OF MILK.




CALCULATE THE NUMBER OF BACTERIA PER GRAM OF HAMBURGER MEAT.
(1 gram = 1ml)




From: http://www.rlc.dcccd.edu/mathsci/reynolds/micro/lab_manual/dilution_prob.html

# More Questions to Answer:

1. One ml of a water sample is added to 9 ml of sterile water. This is mixed well and further diluted by 4 successive 1/10 dilutions. One-tenth of a ml of each dilution is spread on a plate of nutrient agar. After incubation, the following data were obtained:

| Dilution used for plating | Amount plated | Colony counts after incubation |
|---|---|---|
| first 1/10 | 0.1 ml | too many to count |
| second 1/10 | 0.1 ml | 730 |
| third 1/10 | 0.1 ml | 67 |
| fourth 1/10 | 0.1 ml | 5 |
| fifth 1/10 | 0.1 ml | 0 |

**What was the number of colony-forming units/ml of the original water sample? Show all work.**

2. A bacterial culture was diluted and results from duplicate plates were obtained as indicated below.

| Dilution used for plating | Amount plated | Colony counts after incubation (results from duplicate plates) |
|---|---|---|
| $10^{-2}$ | 0.1 ml | too many to count |
| $10^{-3}$ | 0.1 ml | too many to count |
| $10^{-4}$ | 0.1 ml | 321 ; 403 |
| $10^{-5}$ | 0.1 ml | 34 ; 42 |
| $10^{-6}$ | 0.1 ml | 6 ; 1 |
| $10^{-7}$ | 0.1 ml | 0 ; 0 |
| $10^{-8}$ | 0.1 ml | 0 ; 0 |

**What was the number of colony-forming units/ml of the original culture?**

## Spectroscopy

Perform the Virtual spectroscopy lab:

http://bioweb.wku.edu/courses/Biol114/Online/spec20.htm

Questions to answer:

**1. At what protein concentration(s) did you detect maximum absorption?**

**2. At what protein concentration(s) did you detect minimum absorption?**

**3. What is the quantitative relationship between concentration and absorbance?**

Spectroscopy readings can also be performed to determine microbial population counts. Higher cell densities give higher optical densities (O.D.'s), and lower cell densities give lower O.D.'s. Lets assume a value of 1.0 from the spectrophotometer is equivalent to $3x10^8$ cells/ml, i.e., $O.D._{660}$ of 1.0 = $3x10^8$ cells/ml.

**How many cells/ml are in the sample with $O.D._{660}$ = 0.4?**

**By what factor must we reduce a population of $7.2 \times 10^9$ cells/ml to get to 720 cells/ml?**

## Other Dilution problems:

This same discussion applies to any dilution problem whether it is counting cells/ml or making a working solution of 12 mM from a 1 M stock solution – or anything in between. The same principles apply.

To approach a dilution problem (as with any word problem) first determine what information you have been given. There are three big pieces to each dilution puzzle:

1. The Initial concentration (where you start)
2. The final concentration (where you finish)
3. The manipulation that must be made (how to get from start to finish)

When you go into a lab to work, the expectation will be that you are capable of performing these types of calculations. The person for whom you are working will be at least favorably impressed; at best thrilled that you can work on your own to do dilutions.

1. **How would you prepare 500 ml of 3 M HCl using 6 M HCl from the stockroom. Basically, how much water and how much 6 M HCl would you mix to get this dilution? Solve and show work.**

2. **How would you prepare 800 ml of a 3% hydrogen peroxide solution using a 10% $H_2O_2$ solution? Solve and show work.**

Based upon material from: http://members.aol.com/profchm/dilution.html,
http://www.rci.rutgers.edu/~microlab/CLASSINFO/IMAGESCI/doingdilutionproblems.htm