Mathematics Exercises in Biotechnology

Edited by
Mary Ann Hovis
Robert L. Kimball
John C. Peterson

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Preface

AMATYC received supplemental funding from the National Science Foundation (NSF) for the project "Technical Mathematics for Tomorrow: Recommendations and Exemplary Programs." Project recommendations for the original phase were contained in the publication *A Vision: Mathematics for the Emerging Technologies*. Each member of AMATYC received a copy of the *Vision*. Among the recommendations in the *Vision* was a reform in mathematics texts.

Participants felt that textbooks should include writing assignments, projects, technology-based activities, a sufficient amount of skill-and-drill exercises, useful web materials, and information relevant to the technologies represented in their mathematics courses. They also felt that some materials should include too much information and other materials should omit some relevant information and force students to find the missing information.

The materials the participants described are, for the most part, not available in areas of emerging technologies. The additional funding was used to create these types of materials for biotechnology area. The materials were created for the classroom and should reflect the mathematics needed in biotechnology.

The effort involved nine biotechnologists and three mathematicians. Each biotech person developed about 14 problems. Using Bloom's Taxonomy, each problem was classified at one of Bloom's six levels. Each person originally developed at least one problem in each level. A $2\frac{1}{2}$ day workshop was held at Wake Technical Community College, May 16–19, 2004. At this workshop, the biotechnologists interacted with several two-year college mathematics faculty. The purpose of the workshop was to refine the work of each person and make sure that the content was sound, from both a biological and mathematical view, and that the problems were appropriate for the two-year curriculum. At the workshop, several of the problems were merged in order to reduce duplication. Also, many of the anwsers were rewritten to make them more understandable to mathematicians. A glossary at the end of this document will help explain some of the biotech concepts.

The problems were "field tested" with several Wake Tech students. A themed session at the AMATYC Conference in Orlando, Florida highlighted these projects. Authors presented the projects and answered questions about the context.

The project's directors would like to thank the members of the biotechnology community who wrote and edited these problems. We would also like to thank all the participants in the workshop for their hard work and patience. We especially want to thank the students and faculty at Wake Technical Community College who gave us their advice on how to make the problems more understandable to two-year college students. Doris Engler, Rhodes State College, and Monika Collier, Wake Tech, provided valuate clerical support that was appreciated. Finally, we want to thank the National Science Foundation for the funding.

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Project Directors:

Mary Ann Hovis
James A. Rhodes State College, Lima, Ohio

Robert L. Kimball
Wake Technical Community College, Raleigh, North Carolina

John C. Peterson
Chattanooga State Technical Community College, Chattanooga, Tennessee

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Cross-Reference, by Chapter, of Mathematics and Biotechnology Areas

	I. Concentration/Dilution	II. Solution Preparation	III. Serial Dilution	IV. Calibration	V. Molarity	VI. Radioactive Decay	VII. Cell Growth	VIII. DNA	IX. Dosages	X. Beer-Lambert	XI. Absorbtion	XII. Quality Control	XIII. Multi Use
Algebra	4, 6, 7				25, 27		36		41	43, 45			
Cost analysis								38					
Dimensional analysis		16, 20					30, 31	38					
Linear Equations	2							2			50		
Graphs											52		59
Regression	3									44			59
Exponents	11						35						
Exponential Growth and Decay						29		37					
Fractions	7												
Geometry							30						
Graphing	1, 3, 39							39			52	54, 57	59, 60
Logarithms								37			49		
Percent		16, 18, 20					34	38				58	
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Rate and ratio	1, 5, 8, 12	17	5		24		31						60
Scientific notation	11		21				35						
Statistical process control												54	
Statistics				23			35					54, 55, 56, 57, 58	
Unit conversion	2, 7, 11	14, 15, 18	21		24, 26, 28	29	30		40		46, 47, 48		
Units	7	20			25		36		40				
Volume							30						

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Part I

Concentration/Dilution

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1. Concentration of Egg White Lysozyme

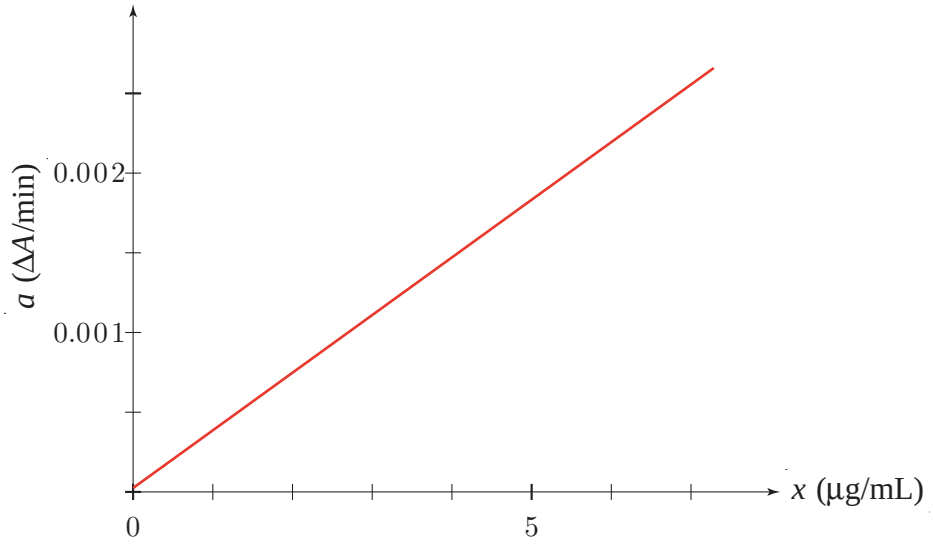
The activity, a, is the rate at which the enzyme converts a colorless substrate to a colored product. Activity is measured as the change in absorbance, A, per minute (ΔA/min) and can be read by a machine called a spectrophotometer. A standard curve for activity of egg white lysozyme at 20°C and 24 ppt salinity has the equation $a = 0.0025x + 0.0005$, where x is concentration in μg/mL. In this equation, the 0.0025 has units of mL/(μg·min) and 0.0005 has units min^{-1}.

(a) Draw a standard curve for this activity.

(b) If a sample of egg white lysozyme has activity of 0.013/min, what is the concentration of that solution, in μg/mL? (Notice that ΔA does not have any units.)

Solution:

(a) The graph of $a == 0.0025x + 0.0005$ is shown in the figure below.



(b) Substituting 0.013/min for a produces

$$\begin{aligned} 0.013/\text{min} &= 0.0025\,\text{mL}/(\mu\text{g}\cdot\text{min})(x\,\mu\text{g/mL}) + 0.0005/\text{min} \\ &= \frac{0.0025\,\text{mL}}{\mu\text{g}\cdot\text{min}} \cdot \frac{x\,\mu\text{g}}{\text{mL}} + 0.0005/\text{min} \\ &= 0.0025x/\text{min} + 0.0005/\text{min} \\ 0.013 - 0.0005 &= 0.0025x \\ 0.0125 &= 0.0025x \\ x &= 5.0 \end{aligned}$$

The concentration this solution is $5.0\,\mu$g/mL.

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2. Restriction Digestion of DNA

You have isolated some DNA and now wish to digest it using a restriction enzyme. You have 3.2 mL of DNA at a concentration of 230 μg/mL as determined by UV spectrophotometry. The restriction enzyme concentration is 2500 units/mL. You plan to run 12 digests each cutting 8 μg DNA with 16 units of the enzyme, as demonstrated in Figure 1.

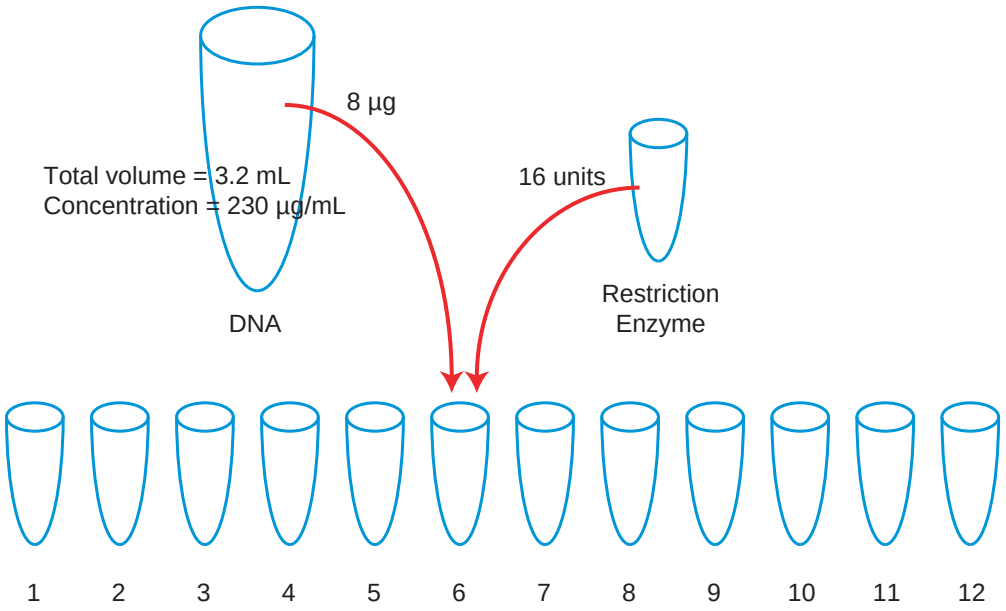

Figure 1

How much of your concentrated DNA and how much enzyme do you need per digest and for all 12 digests? Make sure to check that you actually have enough DNA and enzyme to run the twelve digests. You will need to **(a)** first calculate the amount of DNA required per digest. **(b)** Next, calculate the volume of enzyme required for each digest. **(c)** Finally, calculate the amount of each required for the twelve digests and compare with then amount available.

Solution:

(a) The first step consists of calculating the amount of DNA required per digest. Since each digest consists of 8 μg DNA, we need to determine the number, x of mL of the 8 μg DNA concentrate that will be needed for each digest.

$$\frac{230\,\mu\text{g DNA}}{1\,\text{mL}} \cdot \frac{x\,\text{mL}}{1} = 8\,\mu\text{g DNA}$$

$$x = \frac{8}{230} \approx 0.348\,\text{mL of DNA per digest}$$

Since 1000 μL = 1 mL, this is 34.8 μL. Because of the need for measuring this small quantity, we round this to 35 μL of DNA for each digest.

(b) The next step requires calculating the number of μL of enzyme required per digest.

The amount of enzyme needed per digest is:

$$\frac{2500\text{ units}}{1\,\text{mL}} \cdot \frac{x\,\text{mL}}{1000\,\mu\text{L}} = 16\text{ units}$$

$$\frac{2500x}{1000\,\mu\text{L}} = 16$$

$$2500x = 16\,000\,\mu\text{L}$$

$$x = \frac{16{,}000\,\mu\text{L}}{2500} = 6.4\,\mu\text{L}$$

We have found that 6.4 μL of the enzyme is needed for 16 units to be used in each digest.

(c) For 12 digests, we require $12 \cdot 35\,\mu$L DNA = 420 μL DNA and $12 \cdot 6.4\,\mu$L enzyme = 76.8 μL enzyme.

Since we have isolated 3.2 mL (3200 μL) of DNA, we have enough to carry out the 12 digests. The enzyme is purchased and also needs to be checked to make certain that enough is available.

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3. Using a Standard Graph to Determine the Concentration of an Unknown Solution

The data in Table 1 was collected using a spectrophotometer set at a wavelength of 600 nm.

Table 1

Protein	Absorbance
5 mM	0.24
10 mM	0.30
15 mM	0.44
20 mM	0.48
25 mM	0.59

(a) Plot the five points in Table 1.
(b) Determine the line of best fit through these five points.
(c) Determine the amount of protein present when the absorbance is 0.40.

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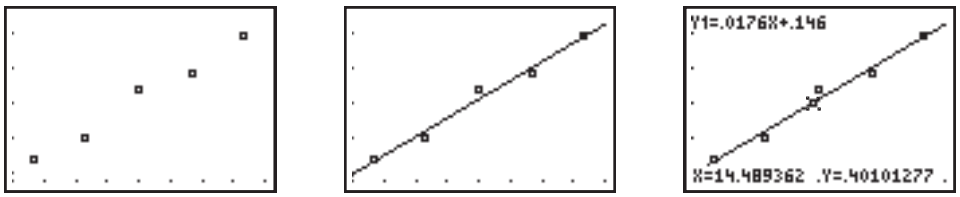
Solution:
The known concentrations are plotted along the x-axis and the resulting absorbance readings are plotted on the y-axis. **Note: You can only plot the first five points since there is no x-coordinate for the last absorbance value.** The resulting graph would look like this:

Using a TI-83 Calculator: Figure 1(a) shows the original five points, Figure 1(b) shows those five points and the regression line $y = 0.0176x + 0.146$, and Figure 1(c) indicates that about 14.48 mM of protein would produce an absorbance of 0.40.



(a) (b) (c)

Figure 1

Using Excel:

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4. Determining the Number of Bacteria in a Culture

Bacteria are commonly used in a wide variety of biotechnology protocols. Many of these procedures require that the number of bacteria in a starting or ending culture be known. A culture of bacteria has been diluted to 1×10^{-6} using a 10-fold serial dilution protocol. Then, 0.1 ml of each of the 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} dilutions was plated onto nutrient agar and 36 hours later the colonies that had formed were counted. The following data was generated:

1×10^{-3} = TNTC (Too Numerous To Count)
1×10^{-4} = TNTC
1×10^{-5} = 237 colonies
1×10^{-6} = 21 colonies

Calculate the estimated number of bacteria as colony forming units (CFU) per milliliter of the original culture. How many bacteria are in each milliliter of the original culture?

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Solution:
The general procedure to determine the number of bacteria in a culture is to dilute the bacterial population of the culture in question using a serial dilution protocol (commonly, 10-fold serial dilution), spread a known volume of the dilutions containing bacteria onto a growth media, such as nutrient agar, and, at some time point after growth has occurred, count the number of colonies that arise on the growth media.

The concept is that each colony represents a single, or possibly a few, bacteria, called a colony forming unit (CFU). Using dilution factors (the inverse of the actual dilution) the technician can then make a determination of the concentration of bacteria in the original culture.

There are three factors that need to be understood by the technician in this procedure. The first is that the spreading of 0.1 ml of a dilution represents a further 1×10^{-1} (1/10) dilution, or reduction, from the 1 ml reporting volume. The second is that the colony count is related to the original number of bacteria by reversing the effects of the plated dilution from which the colony number is obtained. This is accomplished by the use of the dilution factor, which is the inverse of the actual dilution. The final concept is that numerical significance is achieved by a colony count between 50 (enough to be relevant) and 300 (any more and the colonies begin to blend and the counting procedure becomes difficult for humans, leading to errors) colonies.

Solution

The colony count of 237, obtained from the plate with the 1×10^{-5} dilution, meets the criteria of between 50 and 300 colonies. The formula used is

$$\frac{\text{\# of bacteria (CFU)}}{\text{ml in original culture}} = \text{\# of colonies counted} \times \text{ dilution factor for volume plated} \times \text{dilution factor for the dilution of the count being used}$$

For this problem, we have the following information:

\# of colonies counted = 237
Dilution factor (df) for volume plated = 0.1 ml plated = 1/10 ml, therefore, df = 10
Dilution factor (df) for the dilution of the count being used = 1×10^{-5} dilution counted, therefore, df = 1×10^5

Therefore, for this culture,

$$\begin{aligned} \text{\# bacteria (CFU)/ml} &= 237 \text{ colonies} \times 10 \times 1 \times 10^5 \\ &= 2.37 \times 10^8 \end{aligned}$$

Thus, there are 2.37×10^8 bacteria (CFU)/ml in the original culture.

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5. Serial Dilutions

A blood serum sample has been obtained from a patient suspected of being infected with Hepatitis C. A common medical test will be performed to determine **(i)** if the patient is infected and **(ii)** if the patient is infected, roughly where he or she is in the infection cycle based on the amount of antibodies in the blood serum. A low amount of antibodies indicates that the patient is in an early stage and a high amount of antibodies equates to a late stage.

The test will be carried out in a 96-well plate (a square plastic plate with 96 wells in an 8×12 grid). You will need to perform a 2-fold serial dilution to determine the level of the protein antibodies in the blood. The dilutions will be carried out in small tubes and the desired concentrations will be transferred to the proper wells. The procedure for this test requires a minimum volume of 0.2 ml per well across 11 wells in a row. You will also need to set up 3 replicate rows for each patient sample to properly perform the test. Describe in detail how you will set up the dilutions of the patient's blood serum to prepare both the required concentration and volume needed to carry out this test.

(a) Perform a 2-fold serial dilution of the patient's serum that will result in 11 dilutions (with the undiluted, this will be enough diluted samples for one complete row on the 96-well plate) of the required minimum volume.

(b) Determine the actual dilution in each of the 11 tubes.

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Solution:
The steps of the assay beyond those already given are not relevant to this mathematical problem.

(a) Determining the sample volume required for each dilution

Step 1. Each well requires 0.2 ml,

Step 2. Each dilution will require 3 wells (1 well per row, 3 rows for each sample).

Step 3. Each dilution volume will be 0.2 ml/well $\times 3$ wells = 0.6 ml,

Step 4. Therefore, prepare a minimum of 0.75 ml per dilution

(b) Setting up the tubes:

Step 1. A 2-fold dilution series will include 11 dilutions, beginning with undiluted, __, __, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, and 1/2048.

Step 2. Therefore set up 12 tubes labeled 1 through 12.

Step 3. Into tubes #2 through 12, add 0.75 ml of PBS.

Step 4. Place 0.75 ml of sample into tube #1 (undiluted) and into tube #2 (1/2 dilution).

Step 5. Mix the contents of tube #2 and transfer 0.75 ml into tube #3.

Step 6. Mix the contents of tube #3 and transfer 0.75 ml into tube #4.

Step 7. Repeat steps for tubes #4 through #12.

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6. Dilutions of Stock Liquid Solutions

Many of the reagents used in the laboratory are purchased or prepared at high concentrations, called stock solutions, and used at lower concentrations. The ability to "dilute," or weaken the reagent to any desired lower concentration is a standard skill required of all laboratory technicians. You will be required to prepare dilutions of a variety of required concentrations and volumes from stock solutions.

Note: It is often the case that the desired concentration and volume are given while it is the duty of the technician to determine the starting concentration. The starting concentration may be derived from a commercial preparation or from a preparation at a higher concentration not appropriate for the given needs.

From a commercially obtained, concentrated hydrochloric acid you are to prepare 600 ml of a 1.0 M HCl solution.

1. Calculate the amount of stock solution needed.
2. Prepare the solution.

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Solution:
Concentration is a ratio of solute to solvent to yield a total solution. The concept of dilution is that the ratio of the solute to solvent of the original stock solution will be different from the ratio of the solute to the solvent of the new solution, although the solvent and the solution will still be of the same material. Therefore, mathematically, dilutions can be solved by a comparison of the original ration to the resulting ratio. The formula that describes this ratio comparison is $V_1C_1 = V_2C_2$, where

V_1 = the volume of the stock solution used to make the desired dilution,
C_1 = the concentration of the stock solution.
V_2 = the final desired volume of the diluted solution,
C_2 the final desired concentration of the diluted solution, and
M = molar, a unit of concentration equal to one mole of solute per liter of solvent. .

Calculation of amounts needed: Use the equation $V_1C_1 = V_2C_2$ to organize the information, where

V_1 = the required volume of the stock solution,
$C_1 = 12\,\text{M}$ (the concentration of the commercial stock solution),
$V_2 = 1.0\,\text{M}$, the desired concentration of the diluted solution, and
$C_2 = 600\,\text{ml}$, the desired volume of the diluted solution.

Plugging the values into the equation gives

$$\begin{aligned} V_1(12\,\text{M}) &= (500\,\text{ml})(1\,\text{M}) \\ V_1 &= \frac{600\,\text{ml} \times 1\,\text{M}}{12\,\text{M}} \\ V_1 &= 50\,\text{ml of the } 12\,\text{M HCl} \end{aligned}$$

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7. Defining and Distinguishing Between Concentration and Amount of Solute

In each of the five figures below, the stars represent solute. For each figure, fill in the blanks with the amount of solute and the concentration of solute. Express concentration in the units as directed in each question.

(a) In Figure 1 each star represents 1 mg of NaCl.

i. What is the total amount of NaCl in the tube?

ii. What is the concentration of NaCl in the tube (in mg/mL)?

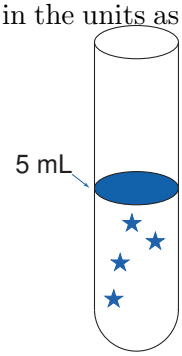

Figure 1

(b) In Figure 2 each star represents 1 millimole of protein kinase.

i. What is the total amount of protein kinase in the tube?

ii. What is the concentration of protein kinase in the tube in millimoles/mL?

iii. What is the concentration of protein kinase in the tube in moles/mL?

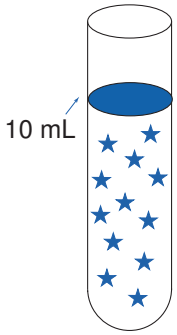

Figure 2

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(c) In Figure 3 each star represents 1 μg of dissolved chlorine.

i. What is the total amount of chlorine in tube?

ii. What is the concentration of chlorine in tube in μg/mL

iii. What is the concentration of chlorine in tube in g/L?

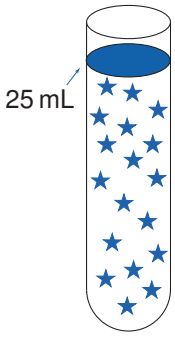

Figure 3

(d) In Figure 4 each star represents 1 mg of NaCl.

i. What is the total amount of NaCl in the container?

ii. What is the concentration of NaCl in the container expressed as a per cent?

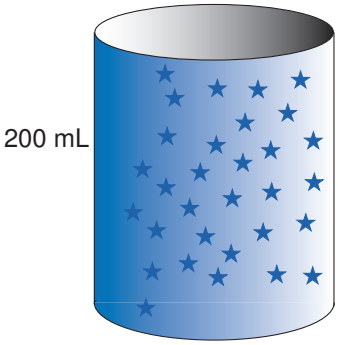

Figure 4

(e) In Figure 5 each star represents 1 mg of dioxin.

i. What is the total amount of dioxin in the container?

ii. What is the concentration of dioxin in the container expressed as ppm (parts per million)?

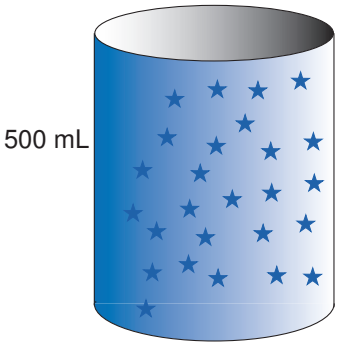

Figure 5

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Solution:

(a) In Figure 1 each star represents 1 mg of NaCl.

i. What is the total amount of NaCl in the tube? 4 mg

ii. What is the concentration of NaCl in the tube (in mg/mL)?

$$\frac{4\,\text{mg}}{5\,\text{mL}} = \frac{?}{1\,\text{mL}}$$
$$? = 0.8\,\text{mg}$$

So, the concentration is 0.8 mg/mL

(b) In Figure 2 each star represents 1 millimole of protein kinase.

i. What is the total amount of protein kinase in the tube? 12 millimoles

ii. What is the concentration of protein kinase in the tube in millimoles/mL? 1.2 millimoles/mL

iii. What is the concentration of protein kinase in the tube in moles/mL? 1.2×10^{-3} moles/mL

(c) In Figure 3 each star represents 1 g of dissolved chlorine.

i. What is the total amount of chlorine in tube? 18 g

ii. What is the concentration of chlorine in tube in μg/mL 0.72 g/mL

iii. What is the concentration of chlorine in tube in g/L? $0.72\,\mu\text{g/mL} = 0.72 \times 10^{-6}\,\text{g/mL} = 0.72 \times 10^{-3}\,\text{g/L} = 7.2 \times 10^{-4}$ g/L

(d) In Figure 4 each star represents 1 mg of NaCl.

i. What is the total amount of NaCl in the container? 30 mg

ii. What is the concentration of NaCl in the container expressed as a per cent? $\frac{30\,\text{mg}}{200\,\text{mL}} = \frac{15\,\text{mg}}{100\,\text{mL}}$. Since 1 mL has a mass of 1 g, then $\frac{15\,\text{mg}}{100\,\text{mL}} = \frac{15\,\text{mg}}{100\,\text{mg}} = 0.15$, and so the concentration is 0.15%.

(e) In Figure 5 each star represents 1 mg of dioxin.

i. What is the total amount of dioxin in the container? 25 mg

ii. What is the concentration of dioxin in the container expressed as ppm (parts per million)? Since 1 mL of water has a mass of 1 g 1 ppm in water $= \frac{1\,\mu\text{g}}{\text{mL}}$. Thus, $\frac{25\,\text{mg}}{500\,\text{mL}} = 0.05\,\text{mg/mL} = 50\,\mu\text{g/mL}$. So the concentration is 50 ppm.

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8. Absorbance and Transmittance in a Spectrophotometer

The absorbance of light by a solution is directly proportional to the concentration of living cells in the solution. If the absorbance of light is measured using an automatic plate reader, then the concentration-absorbance relationship is linear when the absorbance numbers are between 0.1 and 2.0.

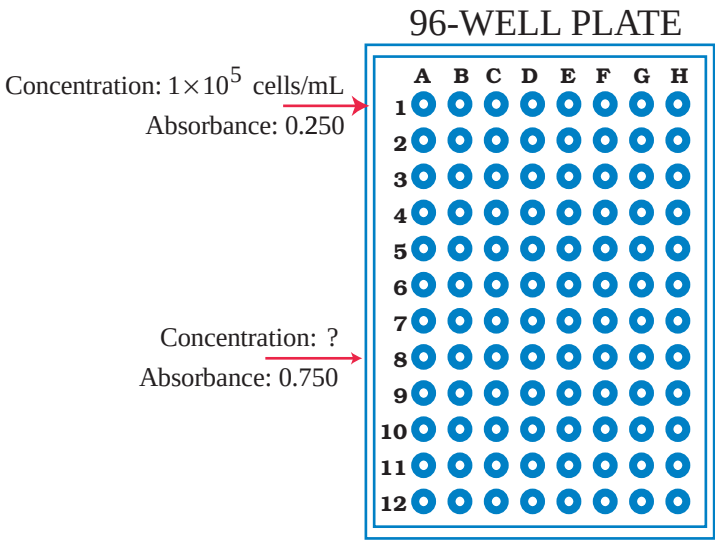

Figure 1

The concentration of cells in well A1 of the 96-well plate in Figure 8 is 1×10^5 cells/mL and its absorbance is 0.250, then what was the approximate concentration in each of the following neighboring wells:

(a) A8, with the absorbance indicated in Figure 8,

(b) Cell C6, with an absorbance of 0.825,

(c) Cell F10, with an absorbance of 1.625, and

(d) Cell D7, with an absorbance of 2.450?

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Solution:

(a) Use the proportion $\dfrac{\text{Absorbance in Cells A}}{\text{Concentration in Cells A}} = \dfrac{\text{Absorbance in Cells B}}{\text{Concentration in Cells B}}$. It does not matter which cell is labeled A and which is called cell B. Here we will let Cell A be the one with an absorbance reading of 0.250 and a concentration of 1×10^5 cells/mL. Thus, Cell B has an absorbance of 0.750. This leads to the following answer:

$$\begin{aligned}
\frac{\text{Absorbance in Well A1}}{\text{Concentration in Well A1}} &= \frac{\text{Absorbance in Well A8}}{\text{Concentration in Well A8}} \\
\frac{0.250}{1 \times 10^5\,\text{cells/mL}} &= \frac{0.750}{x} \\
0.250z &= 0.750 \times 1 \times 10^5\,\text{cells/mL} \\
x &= \frac{0.750 \times 1 \times 10^5\,\text{cells/mL}}{0.250} \\
x &= 3 \times 1 \times 10^5\,\text{cells/mL} \\
x &= 3 \times 10^5\,\text{cells/mL}
\end{aligned}$$

Well A8 has a concentration of 3×10^5 cells/mL.

(b) For Well C6, the proportion is $\dfrac{0.250}{1 \times 10^5\,\text{cells/mL}} = \dfrac{0.825}{x}$. Solving this proportion, results in 3.3×10^5 cells/mL,

(c) Well F10 has a concentration of 6.5×10^5 cells/mL.

(d) The concentration-absorbance relationship is only linear when the absorbance is between 0.1 and 2.0. Thus, the absorbance of 2.450 in Well D7 is meaningless.

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9. Creatine Kinase and Tissue Injury

A certain research laboratory performs the creatine kinase assay (a test) on serum samples to determine myocardial (heart muscle) damage to laboratory test animals in a particular study. The laboratory currently has 32 μL of creatine kinase stock solution. The stock solution of creatine kinase has a concentration of 4000 Units/mL. The assay (the test) requires a final concentration of 2 Units/mL of creatine kinase, and each tube has a total volume of 5 mL (that is, the sum total of all additions to the tube).

(a) How many μL of creatine kinase does each control tube require?

(b) How many assays can you perform before you run out of the purified creatine kinase?

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Solution:

(a) Total activity of creatine kinase per tube = 5 mL × 2 Units/mL = 10 Units.
4000 Units of enzyme activity are present in 1 mL (or 1000 μL) of stock solution.
Therefore, 10 Units are present in 10 Units × 1000 μL ÷ 4000 μL = 2.5 μL.

(b) 1 tube requires 2.5 μL, and the total volume of creatine kinase available is 32 μL. Therefore, the laboratory has enough enzyme for 12 batches of assay (32 μL ÷ 2.5 μL = 12 with a remainder of 2 μL).

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

10. PCR Laboratory Using Custom Primers

An intern at the local crime lab was handed two DNA samples for PCR (Polymerase Chain Reaction) analysis, along with all of the required components for the assay, and the standard procedure for setting up the reaction. The PCR procedure called for 25 pmoles (picomoles) of primer A in a volume of 1 μL, and 25 pmoles of primer B in a volume of 1 μL as well. However, the intern realized that he was given a fresh batch of primers that had not yet been reconstituted in buffer solution. According to the printed labels on the primer vials, there were 92.9 nmoles of primer A, and 63.7 nmoles of primer B. The intern added 929 μL of TE buffer to primer A and 637 μL of TE buffer to primer B.

(a) What was the concentration of the two primers in pmoles/μL in stock A and stock B?

(b) In what proportion should stock A or stock B be added to the buffer solution to attain 25 pmoles/μL concentration?

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Solution:
The PCR procedure is a commonly used technique in forensics laboratories, and employs oligonucleotide (short stretches of molecules called nucleotides) sequences that serve as primers. When custom primers are ordered from a commercial source, the primers are shipped as a lyophilized powder, and require reconstitution in water or an appropriate buffer.

A mole of any compound contains 6.02×10^{23} molecules of the compound. The molecular weight of a compound is the weight in grams of 1 mole of a compound. A nmol (nanomole) is 10^{-9} mole, a pmol (picomole) is 10^{-12} mole, and a μL (microliter) is 10^{-3} mL or 10^{-6} liter.

(a) 1 nmole equals 10^3 pmoles. Therefore, 92 900 pmoles in $929\,\mu\text{L} = 100\,\text{pmol}/\mu\text{L}$. Similarly, 637 pmoles in $637\,\mu\text{L} = 100\,\text{pmol}/\mu\text{L}$.

(b) $100\,\text{pmol}/\mu\text{L} \div 25\,\text{pmol}/\mu\text{L} = 4$. Hence, a four-fold dilution will be required to obtain $25\,\text{pmol}/\mu\text{L}$ of each primer solution.

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11. Cytotoxicity Testing of Anti-Cancer Drugs

For certain types of cancers, it is common practice to first determine the cytotoxicity profile of the candidate drugs on cells obtained from a biopsy sample of the patient's tumor, before selecting a drug for use on the patient. Several research and medical labs provide the cytotoxicity testing as a contract service to area hospitals. In the following scenario, the supervisor asks the technician to set up a 24-well cell culture dish, with each well containing 200 μL of cell culture medium with 1×10^3 cells. The technician first enzymatically breaks up the biopsied tissue and obtains a suspension of cells in culture medium. He then dilutes the cell suspension by adding 90 μL medium to 10 μL cell suspension, and performs a cell count on the diluted sample. Once he determines the total amount of cells obtained, he adjusts the cell concentration to the desired value. The technician obtains a cell count of 48, 56, 45, and 51 for the four grids.

(a) Based on these cell counts, what is the cell concentration of the diluted cell suspension, expressed as number of cells/mL?

(b) What is the cell concentration of the original cell suspension, expressed as number of cells/mL?

(c) How much should he dilute the original cell suspension in order to have 1×10^3 cells in 200 μL?

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Solution:
The hemocytometer contains four counting grids that measure 1mm x 1mm x 0.1mm each. Therefore, the volume of cell suspension counted is predetermined by the configuration of the hemocytometer. A total cell count for the four grids is first obtained and then divided by four to determine the cell count in one grid. It is normally recommended that the total cell count in the four grids does not exceed 300, in order to minimize counting errors that may otherwise result from overlapping cells and from eye strain. Hence it is customary to perform a dilution of the cell suspension prior to counting in the hemocytometer.

(a) Average cell count $= (48 + 56 + 45 + 51) \div 4 = 50$

Volume of each grid $= 1 \times 1 \times 0.1\,\text{mm}^3 = 0.1\,\text{mm}^3$ or $1 \times 10^{-4}\,\text{mL}$ (since $1\ \text{cm}^3 = 1\,\text{mL}$). Therefore, the number of cells in 1 mL can be determined as follows:

$$\frac{50\ \text{cells}}{1 \times 10^{-4}\,\text{mL}} = \frac{?\ \text{cells}}{1\,\text{mL}}$$

$$50\ \text{cells} \times 1\,\text{mL} \div 10^{-4} = 50 \times 10^4\ \text{cells or}\ 5 \times 10^5\ \text{cells/mL}.$$

(b) Since $10\,\mu$L of cell suspension was added to $90\,\mu$L of medium, the dilution factor is $10([10 + 90] \div 10)$. In order to determine the cell concentration of the original cell suspension, we would need to multiply by the dilution factor: $10 \times 5 \times 10^5 = 50 \times 10^5 = 5 \times 10^6$ cells/mL.

(c) The desired final concentration of 1×10^3 cells in $200\,\mu$L equals a concentration of 5×10^3 in 1 mL (since $1\,\text{mL} = 1000, \mu\text{L}$). The dilution factor can be determined by dividing the original concentration by the desired concentration, as follows: 5×10^6 cells/mL $\div\ 5 \times 10^3 = 1 \times 10^3 = 1000$.

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12. Soft agar cloning

Cell cloning is commonly performed in a Jello-like semi-solid support structure made with a solution of a substance called agar, which is allowed to set, just like Jello. This support structure also contains a nutrient solution to provide nourishment for the cells. While the agar solution needs to be sterilized by heating to a high temperature (autoclaving) to render it sterile, the nutrients in the nutrient solution will be destroyed upon autoclaving. Therefore, the agar solution is commonly prepared at a higher concentration in water (agar stock solution), and mixed with a nutrient solution that contains the nutrients at a higher concentration than needed (nutrient stock solution). When the two solutions are mixed together and diluted with sterile water, the agar and the nutrients are both diluted sufficiently to obtain the right concentrations of each.

(a) The agar stock solution has a concentration of 12% agar (that is, 12 grams agar dissolved in water to a final volume of 100 mL solution), and the nutrient stock solution contains twice the amount of all the nutrients, and is referred to as a 2X nutrient solution. Sterile water is also available. What is the ratio in which the agar stock solution, the 2X nutrient solution and water should be mixed, in order to obtain a final concentration of 3% agar, and 1X of the nutrients?

(b) What volume of the 2X nutrient solution and sterile water should be added to 50 mL of the 12% nutrient agar stock solution to obtain the correct final concentration of agar and nutrients?

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:

(a) Since the stock concentration of the agar solution is 12% and the desired final concentration of agar is 3%, the agar stock solution should be diluted 4-fold, that is, 1 part in a total of 4 parts. Since the nutrient solution is 2X and it needs to be at a final concentration of 1X, it should be diluted 2-fold, that is, 1 part in 2 parts or 2 parts in 4 parts.

Therefore, agar stock solution ratio 2X nutrient solution : water is $1 : 2 : 1$.

(b) If 50 mL (1 part) of agar stock solution is used, it should be mixed with 100 mL (2 parts) nutrient stock solution and 50 mL (1 part) of sterile water to get the right final concentrations.

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13. Cell Culture Medium

Cell culture media are commonly supplemented with bovine serum in order to provide the cells with growth factors and hormones that are present in serum. Typically, a 10% solution of bovine serum in the cell culture medium is prepared (that is, 10 mL bovine serum in a total volume of 100 mL). How much bovine serum would need to be added to 500 mL of cell culture medium to obtain a final concentration of 10% bovine serum? You may round the answer to the second decimal place.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:
Cell culture media often come in a quantity of 500 mL. Adding the right amount of serum to the original container of medium is preferred over transferring a measured volume of medium to a different container to mix with serum, as the latter method increases the chances of contamination of the medium.

$$\frac{90\,\text{mL}}{100\,\text{mL}} = \frac{500\,\text{mL}}{?\,\text{mL}}$$
$$? \times 90\,\text{mL} = 100\,\text{mL} \times 500\,\text{mL}$$
$$? = 100\,\text{mL} \times 500\,\text{mL} \div 90\,\text{mL}$$
$$? = 555.56\,\text{mL total volume of medium + serum}$$

You will need 555.56 mL medium+serum –500 mL medium = 55.56 mL serum

Since 90 mL of cell culture medium will be needed to prepare 100 mL of a solution of 10% bovine serum in medium, 500 mL of medium can be used to prepare 555.56 mL of 10% bovine serum in medium. Therefore, 55.56 mL bovine serum can be added to 500 mL of medium.

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Part II

Solution Preparation

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

14. Primer Preparation

(a) You have ordered some DNA and, to your surprise, it arrives freeze-dried. It is labeled "77 nmoles." The technician uses the following procedure to obtain 1.0 mL with a concentration of 3.0 pmoles/μL.

Step 1: Add 1.0 mL of distilled water to the DNA primer. What is the concentration of the result in pmoles/μL?

Step 2: Take 100 μL of the solution made in Step 1 and add 156 μL of distilled water. What is the concentration of the result in pmoles/μL?

Step 3: Take 100 μL of the solution made in Step 2 and add 900 μL of distilled water. What is the concentration of the result in pmoles/μL?

The technician has obtained the desired concentration.

(b) A few days later the technician must use a package of freeze-dried DNA labeled "84 nmoles" to make a concentration of 3.0 pmoles/μL. Describe the process the technician might use following the procedure that was used previously.

(c) A few days after that the technician must use a package of freeze-dried DNA labeled "97 nmoles" to make a concentration of 3.2 pmoles/μL. Describe the process the technician might use following the procedures used previously.

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Solution:

(a) Step 1: Dissolve the DNA primer in 1 mL of distilled water. This gives a concentration of

$$\frac{84\,\text{nmoles}}{1\,\text{mL}} \cdot \frac{1\,\text{mL}}{1000\,\mu\text{L}} \cdot \frac{1000\,\text{pmoles}}{1\,\text{nmoles}} = \frac{84\,\text{pmoles}}{1\,\mu\text{L}}$$

Step 2: Take 100 μL of the solution in Step 1 and add x μL of distilled water to get a concentration of 30 pmoles/μL. Solving this for x results in:

$$\frac{84\,\text{pmoles}}{(100+x)\,\mu\text{L}} = \frac{30\,\text{pmoles}}{1\,\mu\text{L}}$$
$$x = 180$$

The 100 μL of the solution from Step 1, contains are 84 pmoles/μL $\times 100\,\mu$L = 8400 pmoles.

So the concentration is now: 8400 pmoles/(100 μL + 180 μL) = 30 pmoles/μL

Step 3: Take 100 μL of the solution in Step 2 and add 900 μL of distilled water. The 100 μL of the solution in Step 2, has 30 pmoles/ μL $\times$ 100 μL = 3000 pmoles. The concentration is now 3000 pmoles/(100 μL + 900 μL) = 3 pmoles/ μL

Alternate Method:

Use Step 1 to obtain 1 mL with a concentration of 84 pmoles/μL. You want to add x μL of distilled water to this to obtain a final solution of 3 pmoles/μL. To find the value of x, solve the proportion

$$\frac{84\,\text{pmoles}}{(1+x)\,\mu\text{L}} = \frac{3\,\text{pmoles}}{1\,\mu\text{L}}$$
$$\frac{84\,\text{pmoles}}{3\,\text{pmoles}} = \frac{(1+x)\,\mu\text{L}}{1\,\mu\text{L}}$$
$$28 = 1 + x$$
$$27 = x$$

Thus, if you add 27 μL of distilled water to 1μL of distilled water to the solution from Step 1 to obtain a final solution of 3 pmoles/μL.

(b) Step 1: Dissolve the DNA primer in 1 mL of distilled water. This gives a concentration of

$$\frac{97\,\text{nmoles}}{1\,\text{mL}} \cdot \frac{1\,\text{mL}}{1000\,\mu\text{L}} \cdot \frac{1000\,\text{pmoles}}{1\,\text{nmoles}} = \frac{97\,\text{pmoles}}{1\,\mu\text{L}}$$

Step 2: Using the alternate method described above, yields the following result: You want to add x μL of distilled water to this to obtain a final solution of 3.2 pmoles/μL. To find the value of x, solve the proportion

$$\frac{97\,\text{pmoles}}{(1+x)\,\mu\text{L}} = \frac{3.2\,\text{pmoles}}{1\,\mu\text{L}}$$
$$\frac{97\,\text{pmoles}}{3.2\,\text{pmoles}} = \frac{(1+x)\,\mu\text{L}}{1\,\mu\text{L}}$$
$$97\,\text{pmoles} \cdot \mu\text{L} = (3.2 + 3.2x\,\text{pmoles} \cdot \mu\text{L}$$
$$93.8 = 3.2x$$
$$29.3 \approx x$$

Thus, if you add about 29.3 μL of distilled water to 1μL of distilled water to the solution from Step 1 to obtain a final solution of 3.2 pmoles/μL.

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15. Buffer Preparation

Buffer preparation from stock solution, unit conversion, mixture problemsMeg Rawls

You need to prepare 1 L of solution. It will be prepared from a 20X buffer, a solution that it is 20 times more concentrated than what you would normally use for a working solution. To get the final quantity of working solution, you will mix some of the buffer with distilled water. What volumes of stock (buffer) and distilled water should be mixed?

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Solution:
Because you will not need less than a liter of the 20X solution, we will work in milliliters and use 1 L =1000 mL.

Since $\frac{1}{20}$ of the final solution will consist of the stock solution we need $\frac{1000\,\text{mL}}{20} = 50\,\text{mL}$ of stock solution. To determine the amount of distilled water, subtract

1000 mL	total desired volume
- 50 mL	stock
950 mL	distilled water needed

So, 50 mL of stock and 950 mL of distilled water should be mixed to get the 1 L of final solution.

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16. Percent Solution Conversion

You need to prepare 5 mL of a 2% NaCl solution. You already have a stock solution of 10% NaCl that you are asked to use. How many μL of the 10% NaCl solution would you need to make 5 mL of a 2% NaCl solution?

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Solution:
Method 1: Using the equation $C_1V_1 = C_2V_2$. Use the common equation $C_1V_1 = C_2V_2$, where

$$\begin{aligned} C_1 = &\quad \text{the original concentration,} \\ V_1 = &\quad \text{the original volume,} \\ C_2 = &\quad \text{the final concentration, and} \\ V_2 = &\quad \text{the final volume.} \end{aligned}$$

Note that C_1 and C_2 have to have the same units as do V_1 and V_2. The units on both sides of the equation need to be the same.

In this problem, $C_1 = 10\%$, $V_1 = x$, $C_2 = 2\%$, and $V_2 = 5\,\text{mL}$ $(5000\,\mu\text{L})$. Substituting these values in the equation, leads to

$$\begin{aligned} (10\%)(x) &= (2\%)(5000\,\mu\text{L}) \\ x &= \frac{(2\%)(5000\,\mu\text{L})}{10\%} \\ x &= 1000\,\mu\text{L} = 1\,\text{mL} \end{aligned}$$

You will need $1000\,\mu$L of the 10% NaCl solution in order to make 5 mL of a 2% NaCl solution.
Method 2: Using dimensional analysis.

$$\begin{aligned} 10\% \cdot \frac{1\,\text{mL}}{1000\,\mu\text{L}} \cdot \frac{x\,\mu\text{L}}{5\,\text{mL}} &= 2\% \\ \frac{x\,\mu\text{L}}{5\,\text{mL}} &= \frac{2\%}{10\%} \cdot \frac{1000\,\mu\text{L}}{1\,\text{mL}} \\ x\,\mu\text{L} &= \frac{1}{5} \cdot \frac{1000\,\mu\text{L}}{1\,\text{mL}} \cdot 5\,\text{mL} \\ x\,\mu\text{L} &= 1000\,\mu\text{L} \end{aligned}$$

This gives the same result: $1000\,\mu$L of the 10% NaCl solution will be needed in order to make 5 mL of a 2% NaCl solution.

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17. Preparation of % solutions

A common unit used in the preparation of standard reagents used in the laboratory is percent (%) (from the French for "by 100"). Therefore, percent solutions are ratios based on 100. An x% reagent may be defined as x g in 100 ml of solvent (weight to volume), or as x ml in 100 ml of solution (volume to volume), or as x g in 100 g of mixture (weight to weight). In each case the reference is to 100. Therefore, the x% scenario may be described as

Weight/volume = x g/100 ml solvent
Volume/volume = x ml/100 ml solution
Weight/weight = x g/100 g mixture

(a) How many grams of NaOH will be required to prepare 1 liter of a stock solution of 40% NaOH?
(b) From this stock solution prepare 500 ml of 10% NaOH solution.

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Solution:

(a) This is the basic 40% solution preparation which means that we can think of 40% NaOH $= \dfrac{40\,\text{g NaOH}}{100\,\text{ml } H_2O}$.

In order to find the number of grams, x, needed to prepare 1 liter of a stock solution of 40% NaOH, we need to solve a proportion

$$\begin{aligned}\frac{x\,\text{g NaOH}}{1000\,\text{ml } H_2O} &= \frac{40\,\text{g NaOH}}{100\,\text{ml } H_2O}\\ x\,\text{g NaOH} &= \frac{(40\,\text{g NaOH})(1000\,\text{ml } H_2O)}{100\,\text{ml } H_2O}\\ &= 400\,\text{g NaOH}\end{aligned}$$

It will take 400 grams of NaOH to prepare 1 liter of a stock solution of 40% NaOH.

(b) To prepare 500 ml of a 10% solution from the 40% stock solution use the formula $V_1C_1 = V_2C_2$, where $V_1 = x$ ml of 40% solution, $C_1 = 40\%$, $V_2 = 500$ ml, and $C_2 = 10\%$. Substituting these values into the formula and solving for x results in

$$\begin{aligned}(x\,\text{ml})(40\%) &= (500\,\text{ml})(10\%)\\ x\,\text{ml} &= \frac{(500\,\text{ml})(10\%)}{40\%}\\ &= 125\,\text{ml}\end{aligned}$$

It will take 125 ml of the 40% stock solution of NaOH to have 500 ml of a 10% solution of NaOH.

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18. Calculating How Much Vitamin to Add to Nutrient Broth in a Company Workplace

Biotechnology products are often produced by bacteria that are grown in large vats, called fermenters. A particular vitamin must be added to a nutrient broth that is used to grow the bacteria. The vitamin is purchased in bulk as a freeze dried powder. The manufacturer of the vitamin states that the vitamin powder is 81% pure; the rest is an inert filler that has no effect. You are given an older company procedure that calls for 1 ounce of this vitamin in the pure form to be added to 500 gallons of nutrient broth. You are further told that the company's new fermenter holds 1000 liters of broth.

(a) Calculate how much of the vitamin powder will you need to add to the 1000 liters of broth. Remember to compensate for the inert filler.

(b) Check your answer with another student (or a couple more students) to be sure it is correct. You may also be told to check with your supervisor (teacher).

(c) Document your calculations and the answer you obtained in such a way that an auditor or a colleague will be able to tell that you are correct. Remember that your answer (in a real company) will be used by everyone who prepares this nutrient broth in the future.

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Solution:

(a) First, 1 ounce of vitamin is needed for every 500 gallons of broth and it is necessary to make 1000 liters of broth. Convert 500 gallons to liters where 1 gallon = 3.785 L:

$$\frac{1\,\text{gallon}}{3.785\,\text{L}} = \frac{500\,\text{gallons}}{?}$$
$$? = 1892.5\,\text{L}$$

So, one ounce of vitamin is required for every 1,892.5 L of broth. Therefore 1000 L requires:

$$\frac{1\,\text{ounce}}{1892.\,\text{L}} = \frac{?}{1{,}000\,\text{L}}$$
$$? = 0.52840\,\text{ounce}$$

The vitamin is only 81% pure, so it will be necessary to add more than 0.52840 ounces. If the vitamin is 81% pure, then we want:

$$81\%(?) = 0.52840\,\text{ounce}$$
$$\text{or} \qquad 0.81(?) = 0.52840\,\text{ounce}$$
$$? = 0.65235,\text{ounce}$$

0.65235 is the answer in ounces. It is possible to leave the answer in ounces, if the company plans to use a balance that is calibrated in those units. Otherwise, convert the answer to grams:

1 ounce = 28.35 g, so

$$\frac{0.65235\,\text{ounce}}{?} = \frac{1\,\text{ounce}}{28.35,\text{g}}$$
$$? = 18.494\,\text{g}$$

(b) The answer can be cross-checked using the unit canceling method, and remembering to compensate for the inert filler:

$$\frac{1\,\cancel{\text{ounce}}}{500\,\cancel{\text{gallons}}} \times \frac{1\,\cancel{\text{gallon}}}{3.785\,\cancel{L}} \times \frac{28.35\,\text{g}}{1\,\cancel{\text{ounce}}} \times 1000\,\cancel{L} = ?$$
$$? = 14.980\,\text{g}$$
$$\frac{14.980\,\text{g}}{0.81} = 18.494\,\text{g}$$

This is the same answer as above.

(c) Depending on the company's policies, these calculations might be recorded in a lab notebook or on a form. They would be stored in accordance with the company's policies.

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19. Gel electrophoresis reagent

A reagent solution that is commonly used in a procedure called gel electrophoresis is made by mixing acetic acid, methanol and water in the ratio of 1 : 4 : 5. If you find out that you have 92 mL of methanol, but an unlimited amount of acetic acid and water, how much of this reagent solution can you prepare?

How much acetic acid and water will you need to add to 92 mL methanol?

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Solution:
This problem can be solved using a proportion equation:

Since the ratio of methanol in the mixture is 4/10, we have

$$\frac{4}{10} = \frac{92}{?}$$
$$10 \times 92 \div 4 = ?$$
$$? = 230$$

Therefore, with 92 mL methanol, you will be able to make a total volume of 230 mL of reagent solution.

You can once again use the proportion equation to determine how much acetic acid is needed:

$$\frac{1}{10} = \frac{?}{230}$$
$$230 \times 1 \div 10 = 23$$

Therefore, you will need 23 mL of acetic acid. You can either use the proportion equation to determine the amount of water needed, or more simply as follows:

Volume of water needed = total volume of reagent solution – volume of methanol – volume of acetic acid.

Therefore, Volume of water needed = 230 mL − 92 mL − 23 mL OR 115 mL.

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20. One Hundred Percent Correct Each Time: Preparing Solutions for the Biotechnology Industry

Equipment Needed: (suggested but optional)

Two pieces of equipment are suggested:

1. One empty chemical bottle of NaCl with the label shown in Figure 1. The label should indicate the formula weight of the NaCl as 58.44 g. You can cut the label out that is given below and paste it to an empty plastic bottle.

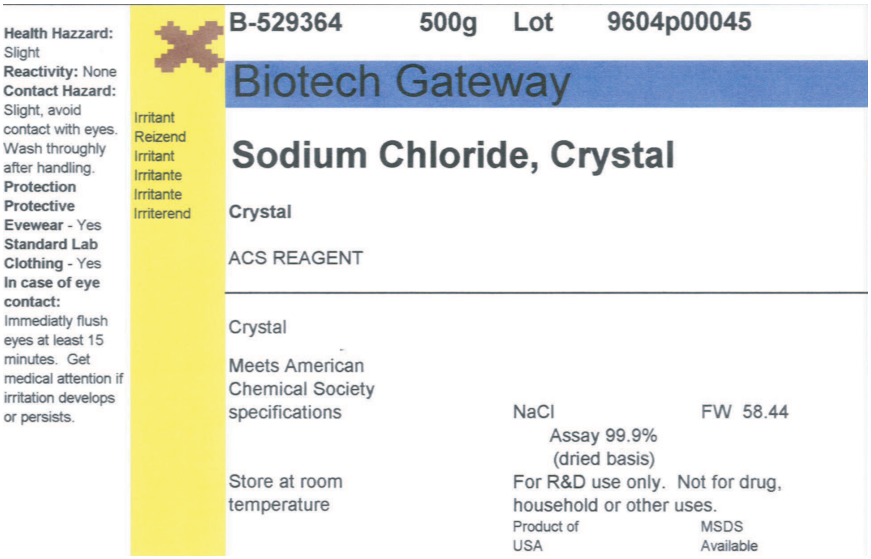

Figure 1

2. A bottle of water with a label

2 M Tris HCl pH 7.2
Prepared by (your name)
Date: (today's date)

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Problem

One of the first questions a biotechnology employer asks of a potential employee is, "Can you prepare solutions?" Not only is a "yes" answer expected, but laboratory supervisors expect their employees to prepare a solution correctly 100% of the time. As part of the interview, the employer will have the interviewee do a problem using the "tools of the trade" to show that he or she can at least do the calculation. For example, the interviewer will give the potential employee an empty chemical bottle of NaCl and a bottle of buffer as indicated, paper, a calculator and the following instruction.

> Using the materials provided (and assume that the bottles are not empty), calculate how you would prepare **(a)** a 2.5 liter solution of 200 mM NaCl and **(b)** 150 mM Tris HCl pH 7.2 . Show all your work.

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Solution:

(a) The formula used to determine the amount of NaCl (X_0 required is X = gram formula weight (fmw) × Molarity desired × Volume desired in liters = 58.44 g/mole × 0.200 mole/liter ×2.5 liters = 29.22 g. Weigh out the salt.

(b) Using the formula $V_1C_1 = V_2C_2$ (where V_1 = volume of stock, C_1 = concentration of stock, V_2 = unknown desired volume from stock, C_2 = known desired concentration) determine the volume of the stock solution 2 M Tris HCl pH 7.2 that is needed to make 2.5 L 150 mM TrisHCl pH 7.2. This means we have the use the following substitutions: $V_1 = X$, $C_1 = 2\,\text{M}$, $V_2 = 2.5\,\text{L}$, and $C_2 = 0.15\,\text{M}$. Putting these into the formula, results in,

$$(X)(2\,\text{M}) = (2.5\,\text{L})(0.15\,\text{M})$$
$$X = 0.1875\,\text{L or } 187.5\,\text{mL}$$

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Part III

Serial Dilution

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21. Saving the Cell Culture

During examination of Chinese Hamster Ovary (CHO) cells kept in culture, the technician discovers that the flask of cells has signs of contamination. Given that the CHO cell line is relatively hardy, she decides to use antibiotics to try and salvage the cells. The stock solution of the antibiotic is 0.1 g/mL, and it needs to be used at a working concentration of 10 μg/mL.

(a) How many 10-fold serial dilutions will need to be performed to reach the working concentration of the antibiotic in the cell culture medium? A 10-fold dilution is 1/10th the concentration of the previous dilution. Each subsequent dilution is made from a previous dilution, so a 10-fold serial dilution is a series of 10-fold dilutions.

(b) The working concentration of the antibiotic is how many orders of magnitude less than the stock concentration?

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Solution:

(a) Stock concentration $= 0.1\,\text{g/mL}$
Working concentration $= 10\,\mu\text{g/mL}$
The first step is to express both concentrations in the same units. $1\,\text{g} = 10^6\,\mu\text{g}$. Therefore, stock concentration $= 0.1x \times 10^6\,\mu\text{g/mL}$ or $10 \times 10^4\,\mu\text{g/mL}$.

Stock concentration $\div$ working concentration $= 10 \times 10^4\,\mu\text{g/mL} \div 10\,\mu\text{g/mL} = 10^4$.

Hence, in order to dilute the stock solution down to $10\,\mu\text{g/mL}$ (or $1 \times 10^1\,\mu\text{g/mL}$), one would need to perform four 10-fold serial dilutions.

(b) The working concentration of the antibiotic is said to be 4 orders of magnitude less than the stock concentration, as one order of magnitude is 10^1.

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22. Test for Endotoxin: A Serial Dilution Problem

Endotoxin is a cell wall component of many bacteria, such as *E. coli*, and is a common water contaminant. Very small amounts of this compound produce fever and other undesirable side effects in humans. As a result, the FDA has mandated that all water that is used to prepare injectable pharmaceuticals must be endotoxin free or at least at such a low level as to not cause a reaction.

The blood of horseshoe crabs clots in the presence of endotoxin. Every year, in Rhode Island, the crabs are collected from the beaches, bled by technicians (see the figure at the right), and returned unharmed to the beaches. Their blood is used to produce the *Limulus Amebocyte Lysate Test* solution (*i.e.*, LAL solution), which is used to quantify the concentration of endotoxin in test samples.

To run the test, the LAL reagent is added to the tubes and the tubes are incubated for 20 minutes. The tubes are then individually flipped over once and checked for the formation of a clot; the solution gels in the bottom of the tube and doesn't run out the tube. The presence of a clot is a positive reaction. The absence of a clot is a negative reaction.

The LAL reagent is considered to be working correctly if the first 4 tubes of control endotoxin (0.5 EU/mL to 0.05 EU/mL) are positive and the endotoxin-free water produces a negative reaction. **Then and only then, are the results obtained with the test samples can be consider VALID!**

(a) Determine how to dilute the endotoxin control reagent (37 EU/mL) to obtain a 2 mL solution with a concentration of 1.0 EU/mL.

(b) You are going to perform a serial dilution using eight 2 mL tubes. Each of the tubes has amount of water indicated in the table below. In tubes 2–8, you take the indicated amount of endotoxin from the previous tube. Determine the endotoxin concentration in each tube.

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Tube #	Water (mL)	Volume of diluted endotoxin added to water	Endotoxin Conc
1	0.0 mL	2.0 mL	
2	1.0 mL	1.0 mL	
3	1.0 mL	1.0 mL	
4	1.2 mL	0.8 mL	
5	1.0 mL	1.0 mL	
6	1.6 mL	0.4 mL	
7	1.0 mL	1.0 mL	
8	1.0 mL	1.0 mL	

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Solution:

(a) The students need to determine the volume of endotoxin control that is needed to set up the controls. In this case, 1.0 mL of 1.0 EU/mL is required so a total of 2 mL should be enough. The concentrated endotoxin is 37 EU/mL. Using the equation $V_1C_1 = V_2C_2$, with $V_1 = x$, $C_1 = 37\,\text{EU/mL}$, $V_2 = 2\,\text{mL}$, and $C_2 = 1\,\text{EU/mL}$, leads to the following solution:

$$x(37\,\text{EU/mL}) = 2\,\text{mL} \times 1\,\text{EU/mL}$$
$$x = 0.054\,\text{mL} = 54\,\mu\text{L}$$

Thus, 0.054 mL or 54 μL of the concentrated endotoxin and 1.9546 mL of endotoxin free water will be needed.

(b)

Tube #	**Water (mL)**	**Vol. of diluted endotoxin added to water**	**Endotoxin Conc**
1	0.0 mL	2.0 mL	1.0 EU/mL
2	1.0 mL	1.0 mL	0.5 EU/mL
3	1.0 mL	1.0 mL	0.25 EU/mL
4	1.2 mL	0.8 mL	0.10 EU/mL
5	1.0 mL	1.0 mL	0.05 EU/mL
6	1.6 mL	0.4 mL	0.02 EU/mL
7	1.0 mL	1.0 mL	0.01 EU/mL
8	1.0 mL	1.0 mL	0.005 EU/mL

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Part IV

Calibration

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23. Monthly Check on Pipetter Accuracy and Precision

Technicians are usually given their own set of digital pipetters: a 10 or 20 μL maximum volume pipetter, a 20–200 μL maximum volume pipetter and a 200–1000 μL maximum volume pipetter. To ensure the accuracy and precision of these pipetters, technicians are required to monthly or in some cases daily, validate these pipetters. It is common for technicians to validate pipetters in the full range of the pipetters, in this case from 200 μL to 1000 μL, to make sure that the pipetter is both accurate and precise at all volumes it is calibrated to measure.

(a) Given the measurements in Table 1, complete the table.

Table 1: Weight of Water Samples

Measurement	**Set to 200 μL**	**Set to 750 μL**	**Set to 1000 μL**
1	0.220 g = 220 μL	0.749 g	1.011 g
2	0.250 g = 250 μL	0.751 g	1.002 g
3	0.180 g = 180 μL	0.747 g	0.997 g
Mean			
Standard deviation (SD)			
Coefficient of variation			
Absolute error			
Percent error			

(b) Use the web, or some other source, and determine the acceptable values for these pepettes.

(c) Base on your findings in **(b)**, if the data indicates the pipette in inaccurate, what are some possible actions you could take?

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Solution:

Optional Equipment Needed:

For accuracy portion, Gilson or similar digital 1000 μL pipetter, a small beaker of water, and a validated and calibrated analytical balance capable of measuring the weight of 100 to 1000 milligrams with a SD of < 0.1 to < 0.2 respectively. For the precision portion, the students only need a pipetter and a small beaker of water.

Equations used:

Mean, $\overline{x}$: The sum of all the values divided by the number of samples.

Standard Deviation, SD: $\sqrt{\frac{\sum(X-\overline{x})^2}{n-1}}$, where X = measurement values , n = number of samples, and $\overline{x}$ = mean

Coefficient of variation (CV): $\frac{\text{Standard deviation}}{\text{mean}} \times 100\%$.

Absolute Error: True Value − Average Measured Value.

Percent Error: $\frac{\text{True Value } - \text{Average Measured Value)}}{\text{True Value}} \times 100\%$.

Table 2: Weight of Water Samples—Completed Table

Measurement	**Set to 200 μL**	**Set to 750 μL**	**Set to 1000 μL**
1	0.220 g = 220 μL	0.749 g	1.011 g
2	0.250 g = 250 μL	0.751 g	1.002 g
3	0.180 g = 180 μL	0.747 g	0.997 g
Mean	217 μL	749 μL	1003 μL
Standard deviation (SD)	35	2	7.1
Coefficient of variation	16%	0.27%	0.71%
Absolute error	−17 μL	1 μL	−3 μL
Percent error	−8.5%	0.13%	−0.3%

The pipetter is not accurate or precise in the lower range in comparison to the middle and higher range of measurement. However, this could also be due to technician error in the handling of the pipetter.

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Part V

Molarity

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24. Salinity Difference

Various saline buffers are used in protein chromatography. Assume that you use one (binding) buffer with a concentration of 4.0 M and a second (elution) buffer with a concentration of 10 mM. By what factor do these two buffers differ in salinity? [Note: The symbol M stands for molarity, a measure of concentration (moles per liter; one mole is a unit of 6.02×10^{23} particles). The term mM means "millimolar" (1/1000th M).]

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Solution:
Converting 10 mM to M we get $10\,\text{mM} = 10 \times 10^{-3}\,\text{M} = 1 \times 10^{-2}\,\text{M}$. The desired factor is determined by dividing: $\dfrac{4.0\,\text{M binding buffer}}{1 \times 10^{-2}\,\text{M elution buffer}} = 400$. The binding buffer is 400 times more saline than the elution buffer.

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25. Reagent Preparation Using Weight to Volume

The most important duty of a lab technician is the preparation of reagents (laboratory solutions used for a number of different purposes) at the correct concentration. No amount of skill in the performance of a laboratory procedure can overcome the use of a poorly prepared reagent, the results will always be wrong!

Using the formula weight and the concepts of moles and molarity, calculate the correct amount of solute to dissolve in the appropriate amount of lab water to prepare 1.5 liters of 1.2 molar calcium chloride solution.

Definitions

Formula weight (FW) is the total weight of all of the atoms in the compound. For example, calcium chloride, $CaCl_2$, has a FW of 111.1 atom mass units (amu)

Mole: One mole of a compound is equal to the formula weight of that compound in grams, aka, the gram molecular weight (gmw). For example, $CaCl_2$ has a gmw of 111.1 g,

Molarity The molarity of a solution is calculated by taking the moles of solute and dividing by the liters of solution. For example, a 1.0 M solution of $CaCl_2$ would contain 111.1 g of $CaCl_2$ in 1 liter of water.

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Solution:

Calculation of amounts needed: The formula used to determine the amount of $CaCl_2$ (x g) required is

$$\begin{aligned} x \text{ g of solute} = {} & \text{gram molecular weight (gmw) of solute} \times \text{Molarity desired} \\ & \times \text{Volume desired in liters} \end{aligned}$$

Note: gram molecular weight is the formula weight of a compound in grams.

$$\begin{aligned} x \text{ g of solute} &= 111.1 \text{ g} \times 1.2 \text{ M} \times 1.5 \text{ L} \\ &= 199.98 \text{ g of } CaCl_2 \end{aligned}$$

Preparation of the solution: To prepare the solution, perform the following three steps.

Step 1. Measure about 1300 ml of lab water

Step 2. Dissolve 199.98 g of $CaCl_2$ in the approximate 1300 ml of lab water.

Step 3. Bring To Volume (BTV = 1500 ml) with lab water.

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26. Purification of Lactate Dehydrogenase (LDH)

Conversion of units, equations, proportions, conversion of unitsKunthavi Natarajan

A student worker in a laboratory is asked to prepare 500 mL of the following Homogenization buffer for the purification of the enzyme lactate dehydrogenase (LDH) from chicken breast muscle.

20 mM Tris-HCl, pH 8.6
1 mM beta-mercaptoethanol (BME)
1 mM PMSF (a protease inhibitor)

The student obtained the molecular weight information for the three ingredients from the label, and used this information to calculate the amount of each substance to weigh out for a 500 mL solution.

Molecular weight of Tris = 121.1 g
Molecular weight of BME = 78.13 g
Molecular weight of PMSF = 174.2 g

However, when he went to weigh BME, he realized that unlike the chemicals Tris and PMSF, BME is a liquid and not a powder.

(a) Calculate the weight of Tris and PMSF needed to make 500 mL of Homogenization buffer.

(b) Calculate the volume of BME needed to provide a final concentration of 1 mM. The density of BME is 1.114 g/mL.

(c) Calculate the molarity of the BME solution.

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Solution:

(a) To determine the amount of Tris needed to make 500 mL of a 20 mM (0.02 M) solution of Tris, first calculate the amount of Tris needed to make 1 liter of a 20 mM solution, by solving the following proportion:

$$\frac{121.1\,\text{g}}{1\,\text{M}} = \frac{?\,\text{g}}{0.02\,\text{M}}$$
$$121.1\,\text{g} \times 0.02\,\text{M} \div 1\,\text{M} = ?\,\text{g}$$
$$?\,\text{g} = 2.422\,\text{g}$$

Now, the following proportion is used to calculate the amount of Tris needed to prepare 500 mL of a 20 mM solution:

$$\frac{2.422\,\text{g}}{1000\,\text{mL}} = \frac{?}{500\,\text{mL}}$$
$$2.422\,\text{g} \times 500\,\text{mL} \div 1000\,\text{mL} = ?$$
$$? = 1.211\,\text{g}$$

Using the same method, calculate the amount of PMSF needed to prepare 500 mL of 1 mM (0.001$\dot{\text{M}}$) PMSF.

$$\frac{174.2\,\text{g}}{1\,\text{M}} = \frac{?\,\text{g}}{0.001\,\text{M}}$$
$$174.2\,\text{g} \times 0.001\,\text{M} \div 1\,\text{M} = ?\,\text{g}$$
$$?\,\text{g} = 0.1742\,\text{g}$$
$$\frac{0.1742\,\text{g}}{1000\,\text{mL}} = \frac{?\,\text{g}}{500\,\text{mL}}$$
$$0.1742\,\text{g} \times 500\,\text{mL} \div 1000\,\text{gmL} = ?\,\text{g}$$
$$?\,\text{g} = 0.0871\,\text{g}$$

(b) Determine the amount of BME needed to prepare 500 mL of a 1 mM solution as follows:

$$\frac{78.13\,\text{g}}{1\,\text{M}} = \frac{?\,\text{g}}{0.001\,\text{M}}$$
$$78.13\,\text{g} \times 0.001\,\text{M} \div 1\,\text{M} = ?\,\text{g}$$
$$?\,\text{g} = 0.0781\,\text{g}$$
$$\frac{0.0781\,\text{g}}{1000\,\text{mL}} = \frac{?\,\text{g}}{500\,\text{mL}}$$
$$0.0781\,\text{g} \times 500\,\text{mL} \div 1000\,\text{mL} = ?\,\text{g}$$
$$?\,\text{g} = 0.0390\,\text{g}$$

From the density of BME, we know that 1.114 g are present in 1 mL. Therefore,

$$\frac{1\,\text{mL}}{1.114\,\text{g}} = \frac{?\,\text{mL}}{0.0390\,\text{g}}$$
$$1\,\text{mL} \times 0.0390\,\text{g} \div 1.114\,\text{g} = ?\,\text{mL}$$
$$?\,\text{mL} = 0.035\,\text{mL OR } 35\mu\text{L}$$

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(c) The density of BME is 1.114 g/mL, which is equal to 1114 grams/1 liter.

Since the gram molecular weight of BME is 78.13 g, we can arrive at the following proportionality equation:

$$\frac{1\,\mathrm{M}}{78.13\,\mathrm{g}} = \frac{?}{1141\,\mathrm{g}}$$

$$1141\,\mathrm{g} \times 1\,\mathrm{M} \div 78.13\,\mathrm{g} = ?$$

$$? = 14.6\,\mathrm{M}$$

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27. Preparation of laboratory solutions

Concentration expressions, using formulasKunthavi Natarajan

Using the formula below, calculate the weight in grams of sodium chloride (solute) required to prepare 200 mL (milliliters) of a 25 mM solution of sodium chloride (gram molecular weight of sodium chloride is 58.44 g).

$$\text{Sodium chloride (solute) required} = \text{Molecular weight} \times \text{Molarity} \times \text{volume}$$

where Molecular weight (MW) = the weight in grams of 1 mole of the solute, Molarity = the molar concentration of the solute expressed in moles/liter, and Volume = the volume of solution, expressed in liters.

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Solution:

- Molecular weight of sodium chloride = 58.44 grams/mole
- Molarity of sodium chloride needed = 25 mM = 25/1000 M = 0.025 M
- Volume of solution = 200 mL = 200/1000 = 0.2 liters
- Solute required = 58.44 grams/mole x 0.025 moles/liter $\times$ 0.2 liters = 0.2922 grams

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28. Do Conversions in Your Head (but document the process if working in industry)

One of the simplest, but most important, mathematical tasks of laboratory technicians is to be able to convert units in a timely manner; however it seems to be one of the easiest tasks to mess up.

Change the label at the right, so that the amounts are in milliMolar, microMolar, or Molar. One Molar is one mole/liter.

0.2 M NaCl
0.15 M Tris HCl pH 7.2

Until you can do the problem in your "head," it is suggested that you write the following information as you do the conversions.

- What information is given?
- What information is being asked?
- What is the conversion factor?
- Do the problem!

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Solution:
The given amounts of 0.2 M and 0.15 M are both in Molars.

For the NaCl, we are given 0.2 M = 0.2 moles/liter. This is converted to mM as follows: $\frac{0.2\,\cancel{\text{mole}}}{1\,\text{liter}} \cdot \frac{1000\,\text{millimoles}}{1.0\,\cancel{\text{mole}}} = \frac{200\,\text{millimoles}}{1\,\text{liter}} = 200$ milliMolar or 200 mM.

For the Tris HCl with pH 7.2, we have the following conversion: $\frac{0.15\,\cancel{\text{mole}}}{1\,\text{liter}} \cdot \frac{1000\,\text{millimoles}}{1.0\,\cancel{\text{mole}}} = \frac{150\,\text{millimoles}}{1\,\text{liter}} = 150$ milliMolar or 150 mM.

The conversion factor can also be expressed as 1×10^3 millimoles/1.0 mole.

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Part VI

Radioactive Decay

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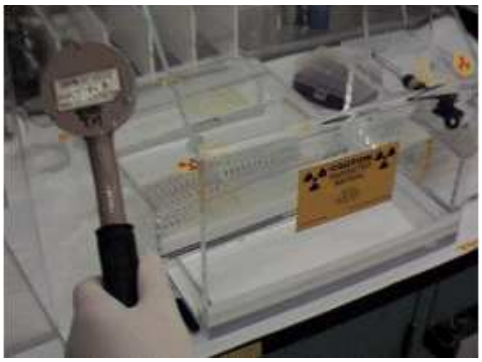
29. Hot Stuff!

A laboratory technician responsible for receiving, logging in and documenting the use of radioactive materials, has just received a shipment of radioactive ^{32}P (a type of phosphorous), ATP (adenosine triphosphate), a total amount of 500 μCi (microCuries), at 30 Ci/mM (millimole) from the company, Atlantic Nuclear.



The technician logs the total activity (500 μCi), sets up the shield, turns on the Geiger counter, puts on two layers of latex gloves, and proceeds to dilute the sample with nonradioactive or "cold" ATP. While doing so, the technician accidentally hits the side of the tube containing the "hot" ATP with her micropipetter, flipping the entire tube onto the floor under the laboratory bench. "Oh my gosh!" screams the technician.

She quickly puts a shield over the spot and reports the spill to the supervisor. The supervisor asks the technician, "Will we be able to remove the shield over the spot in 20 days? You know that in 20 days the President of the school is going to tour the lab, and we can't have the shield over that spot on the floor, we may need to replace that part of the floor. Please figure out if we can remove that shield in 20 days."

The technician knows what the supervisor means when she makes this statement. Essentially, the ^{32}P needs to have decayed to a point that it no longer registers more than environmental background radioactivity as measured by a Geiger counter. The technician also knows that the half-life of radioactive ^{32}P is 14 days.

The Radiation Safety Office has indicated that you can dump 25 μCi per day down the sink. If you wanted to dump the entire shipment of "hot" ATP down the sink, how many days and/or hours would you have to wait until you could actually do it?

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Solution:
Assuming that a safe background radioactivity is $25\,\mu$Ci then the problem could be setup as $25\,\mu\text{Ci} = \left(\frac{1}{2}\right)^{t/14} \times 500\,\mu\text{Ci}$ and solve for t. In this case, $t \approx 60.5$ days, and so, 20 days will NOT be enough time so that the shield can be removed or the this amount of radioactivity could be dumped down the sink.

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Part VII

Cell Growth

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30. Carrot Culture

A piece of carrot the size of a pencil eraser can give rise in tissue culture to a carrot callus with thousands of undifferentiated cells. Theoretically, each cell could give rise to a separate plantlet if transferred to a suitable medium under sterile conditions. Assume that ten plantlets could be grown per 1 mm of carrot root of diameter 2 cm. How many plantlets could be obtained from an carrot root 6 inches long? Assume a constant diameter of 2 cm.

Note: 1 inch = 2.54 cm; 1 cm=10 mm

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Solution:
Begin by converting inches to mm: $6 \cancel{\text{ inches}} \times \frac{2.54 \cancel{\text{ cm}}}{1 \cancel{\text{ inch}}} \times \frac{10 \text{ mm}}{1 \cancel{\text{ cm}}} = 152.4 \text{ mm}.$

Then, multiply to determine the number of plantlets: $152.4 \cancel{\text{ mm}} \times \frac{10 \text{ plantlets}}{1 \cancel{\text{ mm}}} = 1524 \text{ plantlets}.$

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31. Substrate Conversion

If an enzyme attached to a secondary antibody is able to convert 1.25×10^4 molecules of substrate per minute, how many substrate molecules are converted after 90 seconds?

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Solution:
First convert time to minutes: $\frac{90\,\text{seconds}}{1} \times \frac{1\,\text{min}}{60\,\text{seconds}} = 1.5$ minutes. Then, multiple 1.25×10^4 molecules/min $\times 1.5\,\text{min} = 1.875 \times 10^4$ molecules.

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32. Parasite population

Certain cells will double (reproduce) every 32 hours under ideal culture conditions.

(a) If you left a liter of such a cell culture at a concentration of 2.5×10^6 cells/mL on Friday at 5 p.m., calculate the density of cells expected to exist on Monday at 9 a.m.

(b) Assume that on Monday morning you need 100 mL of a working concentration of 5.0×10^5 cells/mL. A biotechnologist has said that in order to prepare this solution, you

- Take 10 mL of the 1.0×10^7 cells/mL stock and add 90 mL of culture broth (diluent).
- Take 50 mL of this solution and add 50 mL of broth.

Explain mathematically why this works.

(c) Now assume that you did not begin work on this project until 1:00 p.m. on Monday. How would you prepare 100 mL of a working concentration of 5.0×10^5 cells/mL?

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Solution:

(a) The time elapsed between Friday at 5 p.m. and Monday at 9 a.m. is 64 hours. This means that there will be $64 \div 32 = 2$ rounds of cell reproduction. Thus, after 64 hours, the cell count should be

$$\begin{aligned} 2.5 \times 10^6 \text{ cells/mL } (\times 2)(\times 2) &= 2.5 \times 10^6 \text{ cells/mL } \times 2^2 \\ &= 1.0 \times 10^7 \text{ cells/mL} \end{aligned}$$

(b)

- Taking 10 mL of the 1.0×10^7 cells/mL stock means that you have a total of $10 \times 1.0 \times 10^7 = 1.0 \times 10^8$ cells. Adding 90 mL of culture broth does not add any cells, so you now have $\dfrac{1.0 \times 10^8 \text{ cells}}{100 \text{ mL}} = 1.0 \times 10^6$ cells/mL.
- Taking 50 mL of this solution gives a total of $50 \times 1.0 \times 10^6 = 5.0 \times 10^7$ cells. Adding 50 mL of culture broth does not add any more cells, so you now have $\dfrac{5.0 \times 10^7 \text{ cells}}{100 \text{ mL}} = 5.0 \times 10^5$ cells/mL.

(c) The time elapsed between Friday at 5 p.m. and Monday at 1 p.m. is 68 hours; $68 \div 32 = 2.125$ rounds of cell reproduction. Thus, after 68 hours, the cell count should be $(2.5 \times 10^6 \text{ cells/mL})\left(2^{2.125}\right) = (2.5 \times 10^6 \text{ cells/mL})\ (4.36) = 1.09 \times 10^7$ cells/mL. Assume that you confirm this count on Monday morning. Your aim is to prepare a working solution of concentration 5×10^5 cells/mL:

$$\begin{aligned} (1.09 \times 10^7 \text{ cells/mL})(?) &= (1.0 \times 10^6 \text{ cells/mL})(100 \text{ mL}) \\ (?) &= \frac{(1.0 \times 10^6 \text{ cells/mL})(100 \text{ mL})}{1.09 \times 10^7 \text{ cells/mL}} \\ (?) &= 9.2 \text{ mL} \end{aligned}$$

Using a graduated pipette, take 9.2 mL of the 1.0×10^7 cells/mL stock and dilute to 100 mL with the culture broth (diluent). This should achieve a concentration of 1.0×10^6 cells/mL. Take 50 mL of this solution and add 50 mL of broth to achieve the final desired concentration.

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33. Transformation Efficiency

Plasmids are small circular pieces of DNA found in bacterial cells. Plasmids are commonly used as vectors to introduce recombinant DNA into host bacterial cells. The process by which this happens is called transformation. Transformation efficiency is a quantitative method that illustrates how many cells are transformed (have taken up the plasmid).

Following a process (called a ligation) by which a unique resistant gene has been inserted into a cloning vector, you have 20 μL of ligation sample containing 0.4 μg of the cloning vector DNA. Two μL of this ligation sample is added to 98 μL sterile water. Ten μL of this dilution is then added to 190 μL competent cells. Following the application of a heat shock, 1000 μL of a growth medium is added to bring the total volume to 1200 μL. After a prescribed incubation, 30 μL are spread on a plate that only allows for the growth of cells containing the cloning vector (transformants). After another 24 hours of incubation at 37°C, there are 133 colonies on the plate. Calculate the transformation efficiency, that is, the number of transformants per μg DNA.

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Solution:

$$\mu\text{g plasmid DNA} = \frac{240\,\mu\text{g}}{2{,}400{,}000} = 1.0 \cdot 10^{-4}$$

$$\text{Transformation efficiency} = \frac{133 \text{ transformants}}{1.0 \cdot 10^{-4}\,\mu\text{g DNA}} \approx 1.33 \cdot 10^{6} \text{ transformants}/\mu\text{g DNA}$$

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

34. Viability Determination

Some cells are counted with a hemacytometer. What is the percent viability of a cell suspension if 130 cells are counted and 22 retain the Trypan blue dye that was added to the cell solution to determine whether the cells were living or dead? Viable cells are living cells and will not take up the stain.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:
Of the 130 cells that were counted 22 retained the Trypan blue dye and thus, were not viable. That means that $130 - 22 = 108$ cells were viable. That means that $\frac{108}{130} \approx 0.831$. Thus, about 83.1% of the cells were viable.

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35. Calculating Cell Density

Vials containing cell suspension were counted using an electronic cell counter. The cell suspension was prepared by adding 0.2 mL of cells into 9.8 mL isotone. (Isotone is a liquid that does not contain any cells.) The counter draws in 1/2 mL for each count and on four successive counts gets 5032, 4992, 5023, and 5019. Using the average (statistical mean) of four counts, calculate the number of cells per mL in the original sample of 0.2 mL.

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Solution:
Average: $\frac{5032+4992+5023+5019}{4} = \frac{20{,}066}{4} = 5016.5$ cells per $1/2\,\text{mL}$. Since the counts each had four significant figures, the average is rounded to 5017 cells per $1/2\,\text{mL}$.

Cells/mL in sample being used for counting: $\dfrac{5017 \text{ cells}}{1/2\,\text{mL}} \times 2 = 10{,}034$ cells.

Cells/mL in oringinal equals cells/mL in counted sample $\times$ dilution factor.

Remember $0.2\,\text{mL}$ of cells were diluted by adding them to $9.8\,\text{mL}$ isotone for a total solution of $10\,\text{mL}$.

The dilution is $\dfrac{0.2\,\text{mL cells}}{10\,\text{mL total}} = \dfrac{1}{50}$, and so the dilution factor is 50.

Cells per mL in original sample $= 10{,}034 \times 50 = 501{,}700 = 5.01 \times 10^5$ cells/mL.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

36. Bacterial Transformation

A DNA transformation procedure was carried out with 0.2 mL of a competent bacterial cell suspension, to which was added 20 ng (nanogram) of plasmid to prepare the transformation mixture. Following this step, 50 μL of the transformation mixture was plated on a LB agar plate containing the antibiotic, ampicillin, to allow the transformed cells to grow.

(a) Given a colony count of 164 colonies, calculate the transformation efficiency per μg of DNA. Transformation efficiency is calculated as the number of colonies resulting from the use of 1 μg of DNA for the transformation experiment.

(b) If the procedure is done right, a transformation efficiency of at least 5×10^3 colonies per μg of DNA can be expected. How many colonies on the agar medium would correspond to this transformation efficiency?

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:
Bacterial cells in suspension, under certain conditions, can take up foreign DNA from solution. Such cells are referred to as "**competent**," and the process of DNA uptake by the cells is called **transformation**. Plasmid is the DNA that is taken up by the bacteria. When bacteria grow on a solid medium called agar, they give rise to colonies or clusters of cells. Only those bacterial cells that have taken up the foreign DNA can grow on the agar medium containing the antibiotic ampicillin, since the foreign DNA confers antibiotic resistance on the transformed bacteria. The colonies are visible on the surface of the agar medium, and can be counted. Each colony arises from a single bacterial cell that has undergone multiple cell divisions. **Transformation efficiency** is calculated as the number of colonies resulting from the use of 1 μg of DNA for the transformation experiment.

(a) 20 ng of plasmid is added to 0.2 mL (200 μL) of bacterial cell suspension. Therefore, 50 μL of cell suspension contains 50 μL $\times$ 20 ng $\div$ 200 μL = 5 ng plasmid.

10^3 ng = 1 μg; therefore, 5 ng = 5 $\div$ 10^3 = 5 $\times$ 10^{-3} μg of plasmid

5 $\times$ 10^{-3} μg of plasmid yields a colony count of 164 colonies. Therefore, 1 μg of plasmid would yield a colony count of 164 colonies $\times$1 μg $\div$ (5 $\times$ 10^{-3} μg) = 32.8 $\times$ 10^3 colonies or 3.28 $\times$ 10^4 colonies

(b) 5 $\times$ 10^3 colonies per μg = 5000 colonies per μg or 5 colonies per ng. Since a total of 5 ng of plasmid was used per agar plate, one would expect 25 colonies on the agar plate.

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Part VIII

DNA

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37. A Case for CSI: Evidence in an Arizona Murder

Hikers found a young woman who had been murdered in the mountains of Arizona. Her partner, a young man from Los Angeles, was charged with the murder, but claimed to be innocent, stating that he was fishing in Michigan at the time. However, seeds were found in his car and were suspected to be from the bristlecone pine, a tree that grows only in the mountains of the southwestern U.S. If the seeds could be analyzed and proven to be from the bristlecone pine, the prosecutor would have a strong case against this man.

To verify the nature of the seeds, DNA was extracted and purified, to be used in analysis. Unfortunately only a tiny amount was obtained. Fortunately, biotechnicians at the crime lab were aware of a widely-used technique called the polymerase chain reaction (PCR). This allows a DNA molecule to be repeatedly doubled. Theoretically, if you could start with a single DNA molecule, after one round of PCR you would have 2 molecules, which, after one more round of PCR would become 4 molecules, and so forth. This, the technicians realized, was a valuable tool in this case, where only a small amount of DNA is available for study. To have enough to analyze, the lab technician had to have at least a billion molecules of DNA.

The DNA sample from the seeds was amplified to allow a larger volume of DNA to be analyzed. If the technician had only a single molecule of purified seed DNA, how many rounds of PCR would be necessary for her to perform in order to obtain at least a billion copies of the molecule? (As mentioned above, each round doubles the number of DNA molecules.)

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:
Each time something is doubled, it is multiplied by 2. Something that is doubled 3 times would have $2 \times 2 \times 2 = 2^3$ the original amount. Something that is doubled n times would have 2^n of the original amount.

In order to determine how many times an original quantity of one has to be doubled to reach one billion, or 10^9, we solve the equation $2^n = 10^9$, where n is the number of rounds of doubling.

$$\begin{aligned} 2^n &= 10^9 \\ \log\left(2^n\right) &= \log\left(10^9\right) \\ n\log\left(2\right) &= \log\left(10^9\right) \\ n\log\left(2\right) &= 9 \\ n &= \frac{9}{\log 2} \approx 29.897 \text{ which rounds up to } 30 \end{aligned}$$

In this case, 30 rounds of PCR would yield approximately a billion DNA molecules, which now allows sufficient DNA for further analysis.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

38. Cost Savings of DNA Sequencing Miniaturization

DNA is the molecular carrier of the genetic code in all living things. It is made up of a sequence of bases that pair to form a double helix. It is now common to determine the sequences. A number of methods have been developed to sequence DNA. Recently, the Sanger Institute developed a method that uses only 1 μL of DNA in tiny containers of a 386 well plate. That means that very little DNA is required and many tests can be run simultaneously. [In fact, 1 μL = $\frac{1}{30}$ drop. There are 60 drops in 1 teaspoon.]

As a lab technician in a biotechnology firm, your employer is considering moving from a sequencing method that requires 10 μL of DNA per test to the Sanger method that uses 1 μL of DNA per text. A team is considering cost savings. Your assignment is to calculate the yearly savings in media (DNA) for running 5,000 samples per day. The company operates both volume and percent. The company operates 250 days during the year. Other team members will be responsible for determining other costs such as sealing materials for the new 386 well plates and other expenses.

(a) How much media is used each year with the present system?

(b) How much media will be used each year if the Sanger method is adopted?

(c) What are the annual volume savings if the Sanger method is adopted?

(d) What are the annual savings, expressed as a percent, if the Sanger method is used rather than the present method?

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Solution:

(a) Using $10\,\mu$L on DNA per sample, the total is $1{,}250{,}000 \cdot 10 = 12,500,000\,\mu\text{L} = 12{,}500\,\text{mL}$ of DNA each year.

(b) By using $1\,\mu$L of DNA per sample, the the company will use $1{,}250{,}000\,\mu\text{L} = 1,250\,\text{mL}$ of DNA each year.

(c) The volume of sample saved is $12{,}500\,\text{mL} - 1{,}250\,\text{mL} = 11{,}250\,\text{mL} = 11.25\,\text{L}$ of DNA per year.

(d) Converting this to a percentage, the savings is $\dfrac{11{,}250\,\text{mL}}{12{,}500\,\text{mL}} = 0.90 = 90\%$.

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39. Corn Chip Crisis! Have these Chips Been Made from Genetically Modified Corn?

There is much controversy over the use of modern biotechnology methods to genetically modify crops used for food production. This activity shows a method that is used to test commercially processed foods to see whether or not they were produced from genetically modified crops and simulates an actual situation that occurred in 1995. At that time, the Terra Prima organic corn chip company in Hudson, Wisconsin discovered that their non-genetically modified corn had apparently been contaminated with corn pollen from a modified Novartis crop planted more than a quarter mile away. Genetic testing showed that pollen from the Novartis crop had drifted to the Terra Prima fields thus contaminating their corn. The Terra Prima Company recalled 90,000 bags of chips, which was a significant monetary loss to a small compan[1]

Suppose that you are an analyst working for the GoodNatured Organic Food Company. A competitor has accused your company of using corn that is genetically modified. You are confident that your company has not intentionally used any genetically modified corn, but you heard about the case involving Terra Prima and want to check for contamination from other fields. Therefore, you obtain 4 bags of your various products, Tortichips, Hotchips, Salsachips, and Bigchips, for analysis. This analysis involves the following seven steps:

1. Isolate the DNA from the chips in each bag, resulting in four samples.

2. Process the DNA from the four samples to look for the special promoter DNA that indicates genetic modification. (See the background information below for information about the promoter DNA and how the DNA is processed.)

3. Run the resulting four samples on an agarose gel. Running the DNA on the gel separates DNA fragments according to their sizes. Each sample is applied to a separate lane on the gel.

4. In addition to the four samples, a lane is run that contains DNA standard marker fragments of known sizes. These standards are necessary to determine the sizes of any DNA fragments observed in the sample lanes.

5. In addition, two lanes are run that do not contain any sample DNA. These two lanes are negative controls – no DNA should appear in these lanes.

6. In addition, another lane is also run as a positive control. This lane contains the special promoter DNA.

[1]Ramanujan, K. and Blum, D. "Genetically modified food 101." *Wisconsin Academy Review*, 46(4), 49-50, 2000.

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7. Stain the gel to make the DNA visible as blue bands. A photograph of the resulting gel is shown in Figure 39.

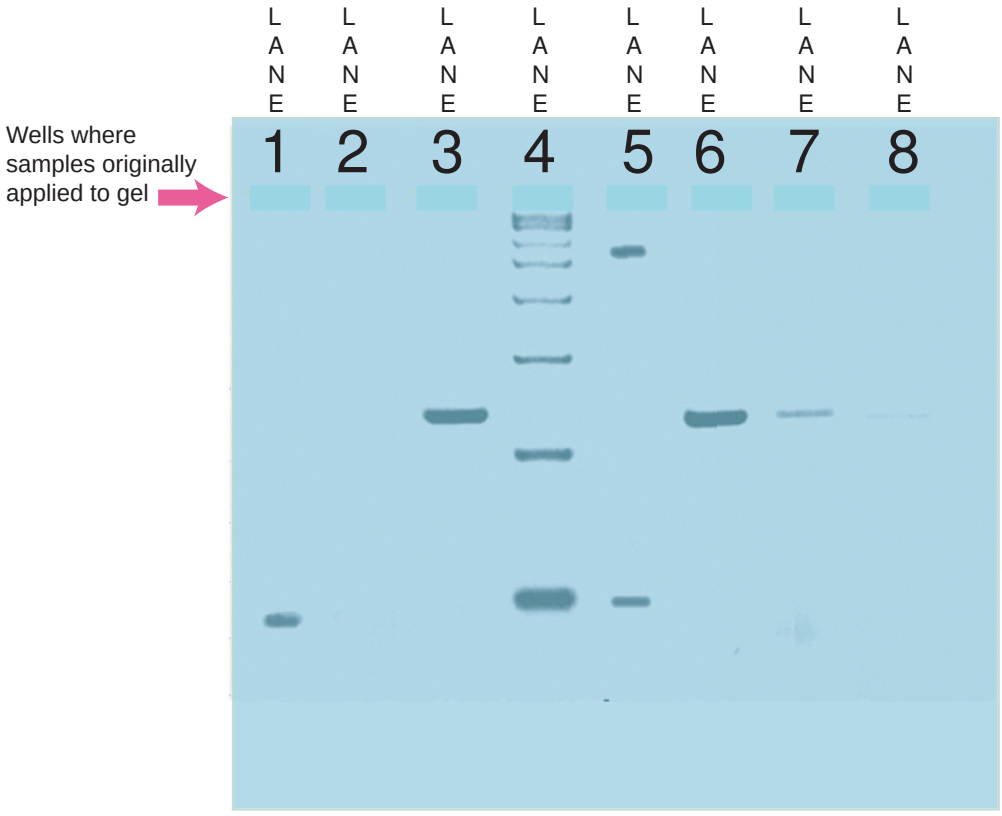

Figure 1

LANE 1: Negative control, sample contains no DNA from any product and so should have no bands

LANE 2: Another negative control

LANE 3: Positive control; contains promoter DNA

LANE 4: Standard Markers, bottom to top: 50 base pairs in length (bp), 150 bp, 300 bp, 500 bp, 750 bp, 1000 bp, 1500 bp, 2000 bp.

LANE 5: DNA from Tortichips

LANE 6: DNA from Hotchips

LANE 7: DNA from Salsachips

LANE 8: DNA from Bigchips

ANALYSIS OF THE RESULTS: The task here is to determine whether or not the GoodNatured cornchips contain genetically modified corn by looking for a band of DNA that is 195 base pairs in length. This band, if present, is likely the special promoter DNA that is introduced into plants when they are genetically modified. DNA standard markers of known length are run in Lane 4

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to which the lengths of bands in the samples can be compared. In principle, we would expect to see DNA bands only in the marker lane, the positive control lane, and in samples where the special promoter DNA was present. This is because the promoter DNA was copied so many times that any other DNA is present in such low concentrations that it is undetectable. In practice, it is sometimes possible to get some faint bands due to non-specific copying of DNA. Bands due to nonspecific copying can be any length. It is also fairly common to see some bands that are below 50 base pairs in size and that are artifacts due to the PCR reaction.

The analysis:

(a) Fill in Table 1 for the DNA standard marker fragments. To do so, carefully measure how far each band in Lane 4 migrated. Measure the distance in millimeters from the bottom edge of the well to the bottom edge of each band.

Table 1

Distance Migrated (mm)								
Length of DNA Fragment (bp)	2000	1500	1000	750	500	300	150	50

(b) Graph your data from the table above on semilog paper as follows:

The x-axis (linear) is the distance migrated and the y-axis (logarithmic) is the base-pair length. How many cycles do you need?

(c) Draw a best fit line through the points.

(d) Determine the base-pair length for all of the bands in the sample lanes by finding their distance migrated on the x-axis. With a ruler, extend a vertical line from this point to its intersection with the best-fit data line. Read across to the y-axis to determine the length of the DNA in that band.

(e) Once you have figured out the size of every band from every sample, decide whether or not your products have likely been contaminated with corn pollen from a genetically-modified crop.

(f) Discuss your results. What size is each band? Are there any bands that appear to be the promoter? Are there any bands that appear to be small artifacts? Are there any bands that appear to be nonspecific DNA? If there are bands that appear to be promoter DNA, discuss what you think the company should do next.

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Part IX

Dosages

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40. Botox Dilemma

The bacterial toxin botulinum, which causes a type of food poisoning, is six million times more toxic than rattlesnake venom. It attaches to nerve endings and so paralyzes muscles. Exposure to a few micrograms of toxin causes death, generally due to paralysis of chest muscles. The toxin can, however, be used to treat patients with a rare disorder, blepharospasm. These patients are blind because their eyes are squeezed shut. Minute doses of the botulinum toxin (Botox) allows patients to open their eyes. In 1989, Botox was approved by the FDA to treat crossed eyes and uncontrollable blinking. Botox has also been used recently to prevent cramps in musicians, calm the spasms of stroke and cerebral palsy, treat migraines, prevent sweating and overactive bladder, and various other therapeutic applications[1][2]. Physicians administering Botox for to treat disorders found that a side effect of the drug is that it erases fine wrinkles around the eyes by relaxing the muscles. Now, the most popular application of Botox is as an alternative to cosmetic surgery.

With Botox, proper application and proper dosage is tricky, but critical. Obviously, at high doses the drug is deadly. But, the drug can have adverse effects at much lower doses. When used for cosmetic purposes, the drug can paralyze the muscles that allow the face to express emotions. In fact, directors such as Martin Scorsese and Baz Luhrmann have complained that some actresses are so heavily botoxed that they can no longer express emotions on camera[3]. Injecting the drug at exactly the right dose and to exactly the right locations is an art as well as a science.

According to the 2003 Physician's Desk Reference (a compendium of information about drugs published by Thomson Healthcare Products), Botox comes in vials of 100 U. (U stands for "units"; the definition of a "unit" is based on how much toxin is required to kill mice.) The specific activity of the drug is 20 U/nanogram of botulinum toxin protein. For treating disease, a typical dose is 200 U total.

The drug is diluted in 1 or 2 mL of sterile saline, and usually is injected 0.1 mL at a time.

According to the medical literature, various studies of Botox for cosmetic purposes have involved total doses between 25 and 50 Units, administered usually in multiple muscle sites (for example, eight injections).

Consider the following hypothetical situation:

> Billy Action, the star of the TV unreality series "Disaster," was fired by the director who claimed that he had lost the ability to express emotions, such as terror, fear, and dismay, and he had begun to slur words. Billy sued his physician, who, he claimed had injected him with too much Botox. An excerpt from Billy's medical file is shown in the box.

[1] Kalish, Nancy. "Beyond Botox," *Health*, pp. 119–124, May, 2003.
[2] Devitt, Terry. "Botox Boom," *On Wisconsin*, pp. 14–15, Spring, 2004.
[3] Devitt, *op. cit.*

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> A vial of Botox cosmetic (manufactured by Allergan) was opened and diluted with 1 mL sterile saline. 0.1 mL was injected into three sites in the upper brow, right quadrant and three sites in the upper brow, left quadrant. 0.05 mL was injected into three sites in the lower right jaw, and three sites in the lower left jaw.

Based on the physician's notes:

(a) What was Billy's total dose of Botox in "units"?

(b) What was Billy's total dose of Botox in nanograms of botulinum toxin?

(c) A few micrograms of this product will cause death. How close was the dose Billy received to this fatal dose?

(d) What do you think about the dose he received and his lawsuit? (You may wish to visit PubMed and search for information about dose and Botox Cosmetic.)

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Solution:

(a) Total dose in Units:

$$\text{Upper face: } 6 \text{ injections} \times \frac{0.1\,\text{mL}}{\text{injection}} \times \frac{100\,\text{U}}{\text{mL}} = 60\,\text{U}.$$

$$\text{Lower face: } 6 \text{ injections} \times \frac{0.05\,\text{mL}}{\text{injection}} \times \frac{100\,\text{U}}{\text{mL}} = 30\,\text{U}.$$

So, the total was 90 Units.

(b) The specific activity is 20 U/nanogram of butulinum toxin:

$$\frac{20 \text{ units}}{1 \text{ nanogram toxin}} = \frac{90 \text{ units}}{?}$$

$$? = 4.5\,\text{nanogram}$$

(c) A microgram is a very small amount, but a nanogram is smaller still—there are 1000 nanograms in a microgram. So, Billy's dose was at least 220 times less than a lethal dose (4.5 nanograms $\times$ 220 = 990 nanograms = 0.990 micrograms).

(d) The dose that Billy received is below the 200 units that is typical when patients are treated for various disorders. It is, however, higher than is typical for cosmetic applications. This does not appear to be a clear cut case and there is likely to be difference of opinion as to whether the doctor was remiss. The class may have various interpretations of the evidence.

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41. Therapeutic Dose of Coffee

Caffeine is found in a variety of foods such as coffee, tea, colas, milk chocolate and in drugs such as Anacin and No-doz. Table 1 shows the amount of caffeine in several popular consumable items.

The most common use for caffeine is that it keeps people awake. It has also been reported that caffeine is an ergonomic aid, and many athletes use it accordingly. Studies have shown that it increases the endurance of many athletes as it promotes the burning of fatty acids sparing glucose. However there is an optimum dose and above that amount, caffeine can cause problems such as increased nervousness, speed up the heart rate, and increase blood pressure. A urine test on athletes that results in a caffeine level of 100 mg/mL or more will result in disqualification. Research has also demonstrated that women of childbearing age and children need more specific guidance regarding their risk and curbing their caffeine intake. It is suggested that reproductive-aged women should consume less than 300 milligrams caffeine daily or 4.6 mg/kg body weight. So what is the optimum dose? Several reports indicate that 200 mg is best; however, in the end, they all say the optimum dose will depend on the individual.

Table 1: Amount of Caffeine Reported in Popular Consumable Items

Drip-brewed coffee	100 mg/ 6 ounce cup
Brewed tea	70 mg/6 ounce cup
Colas (Coke, Pepsi, Mountain Dew)	50 mg/12 ounce
Drugs (Anacin, No-Doz etc.)	Read label
Milk Chocolate	Typically 6 mg per ounce (read label)

Answer the following question: "Based on the amount of caffeine (mg) per kg body weight consumed and your physiological and mental reaction to this amount, do you feel that this is your optimum dose for mental and physical alertness?"

Document all caffeine containing items that you consume in one day or several days. Table 1 indicates several caffeine-containing items that people commonly consume. Notice that both drugs and food items contain caffeine and therefore you may want to pay more attention to the list of ingredients when you are compiling this list.

If you are instructed to do this for several days, it is suggested that you experiment with your dose of caffeine. Try decreasing and increasing your dose per day and, besides documenting the items and amount of caffeine, also document your physical and mental response. Of course, caffeine may not be the root cause of all changes in your well-being but you may notice a trend depending on the amount of caffeine you ingest. Once you have finished documenting these items, determine the amount of caffeine (mg) you are consuming per kg body weight. You may have to convert from pounds to kilograms.

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Solution:
Answers will vary. For example, a student weighs 140 lb has a mass of $140\,\text{lb} \times \dfrac{1\,\text{kg}}{2.205\,\text{lb}} = 308.7\,\text{kg}$. If the student's total caffeine intake was 460 mg, then the caffefine per kilogram was $\dfrac{460\,\text{mg}}{308.7\,\text{kg}} = 1.49\,\text{mg/ kg}$. The student reports that this amount does make her jittery and that when she only drinks two cups of coffee or $200\,\text{mg}/308.7\,\text{kg} = 0.65\,\text{mg/kg}$.

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Part X

Beer-Lambert

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42. Plate Reader Problem

The absorbance of light by a solution is directly proportional to the concentration of living cells, measured in cells/mL, in the solution. If the absorbance of light is measured using an automatic plate reader, then the concentration-absorbance relationship is linear when the absorbance numbers are between 0.1 and 2.0. If the concentration of cells in one well of a 96-well plate was 1×10^5 cells/mL and its absorbance, as determined by a plate reader, was 0.250, then what was the approximate concentration in a neighboring well with an absorbance reading of **(a)** 0.750, **(b)** 0.825, and **(c)** 1.625?

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Solution:

(a) Use the proportion $\dfrac{\text{Absorbance in Cell A}}{\text{Concentration in Cell A}} = \dfrac{\text{Absorbance in Cell B}}{\text{Concentration in Cell B}}$. It does not matter which cell is labeled A and which is called cell B. Here we will let Cell A be the one with an absorbance reading of 0.250 and a concentration of 1×10^5 cells/mL. Thus, Cell B has an absorbance of 0.750. This leads to the following answer:

$$\begin{aligned}
\frac{\text{Absorbance in Cells A}}{\text{Concentration in Cells A}} &= \frac{\text{Absorbance in Cells B}}{\text{Concentration in Cells B}} \\
\frac{0.250}{1 \times 10^5\,\text{cells/mL}} &= \frac{0.750}{x} \\
0.250z &= 0.750 \times 1 \times 10^5\,\text{cells/mL} \\
x &= \frac{0.750 \times 1 \times 10^5\,\text{cells/mL}}{0.250} \\
x &= 3 \times 1 \times 10^5\,\text{cells/mL} \\
x &= 3 \times 10^5\,\text{cells/mL}
\end{aligned}$$

Cell B has a concentration of 3×10^5 cells/mL.

(b) Here the proportion is $\dfrac{0.250}{1 \times 10^5\,\text{cells/mL}} = \dfrac{0.825}{x}$. Solving this proportion, results in 3.3×10^5 cells/mL,

(c) 6.5×10^5 cells/mL.

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43. Using Beer's Law to Determine the Concentration of an Unknown Solution

The following data was collected using a spectrophotometer set at a wavelength of 600 nm.

Table 1

[Protein]	Absorbance
25 mM	0.60
Unknown	0.40

Using the data presented and Beer's law calculate the actual concentration of the unknown solution.

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Solution:
Beer's law defines the relationship between the concentration of a compound and its absorbance at a specific wavelength. Beer's law is expressed as $A = Ma \times C \times L$, where A is the absorbance; Ma the Molar absorbtivity, a constant; C is the concentration, and L is the length of the light path through the sample.

Ma is a constant value that may be obtained from a reference source. L also tends to be constant, usually 1 cm. As constants they can be eliminated from the formula so that all that remains is the concept that the absorbance is proportional to the concentration. Where A_k = absorbance of the known concentration and A_u = absorbance of the unknown concentration, C_k = the concentration of the known solution and C_u is the unknown concentration. From this information the formula for determining the concentration of an unknown compared to a known is $\dfrac{A_u}{A_k} = \dfrac{C_u}{C_k}$.

Using the above formula and given data

$$\begin{aligned} \frac{0.40}{0.60} &= \frac{x}{25\,\text{mM}} \\ x &= \frac{(0.40)(25\,\text{mM})}{0.60} \\ &= \frac{10\,\text{mM}}{0.60} \\ &\approx 16.67\,\text{mM} \end{aligned}$$

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44. The Beer-Lambert Equation: Every Tech Uses It!

An isolated, pure protein is composed of amino acids with a particular structure which confers on its specific physical and chemical properties, one of which is how it absorbs light. In fact, if you did a spectrum analysis of a pure protein in water, it would, EVERY TIME, produce the same absorption curve.

Looking at the absorption curve, you'll see that this protein strongly absorbs light at 280 nm; this is a common trait among proteins. In fact every protein characteristically absorbs a certain amount of light at 280 nm so that, if you measure this absorbance, you could figure out its concentration; the higher the absorbance, the greater the concentration. The relationship between absorbance and concentration is shown in the following equation: $A = Elc$ where A equals absorbance, l is the distance the light has to travel through the sample otherwise known as the path length of the sample tube (Note: this value is usually a constant is equal to 1 cm), and c is the concentration of the sample commonly given in mg per mL. The last variable is E or extinction coefficient, which is described as a constant for that particular protein. It describes how much light is absorbed at a particular wavelength for that specific protein at a particular concentration.

Machines commonly used to measure the absorption of a sample are known as colorimeters or spectrophotometers.

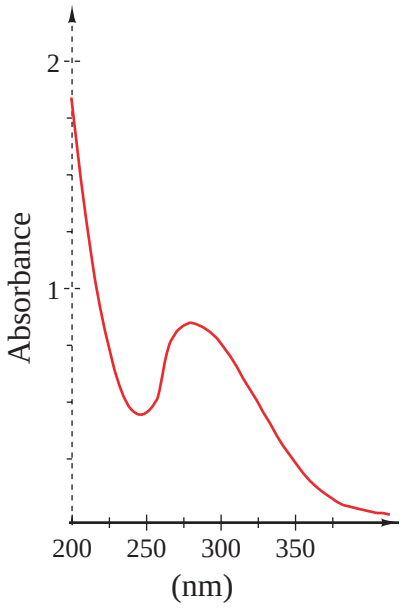

Figure 1

You have been given a sample of what is believed to be an isolated pure protein from a space mission to Mars. It has been determined that it is soluble in water. For your safety, it has been decontaminated.

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The role of the protein is still unknown but it was isolated from a soil sample taken from what is believed to be an ancient riverbed. You are given the task of determining some of its physical and chemical properties, one of which is its E value. Like so many other proteins, it produces a maximal absorption peak at 280 nm.

Given this protein, a spectrophotometer, and was used and the data in Table 1, on the next page, was obtained. Determine the value of E in the equation $A = Elc$.

Table 1

Concentration	**Absorbance Value 280 nm**
1.0 mg/mL	0.91
0.8 mg/mL	0.65
0.6 mg/mL	0.48
0.4 mg/mL	0.26
0.2 mg/mL	0.11
0	0

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Solution:

Concentration	Absorbance Value 280 nm
1.0 mg/mL	0.91
0.8 mg/mL	0.65
0.6 mg/mL	0.48
0.4 mg/mL	0.26
0.2 mg/mL	0.11
0	0

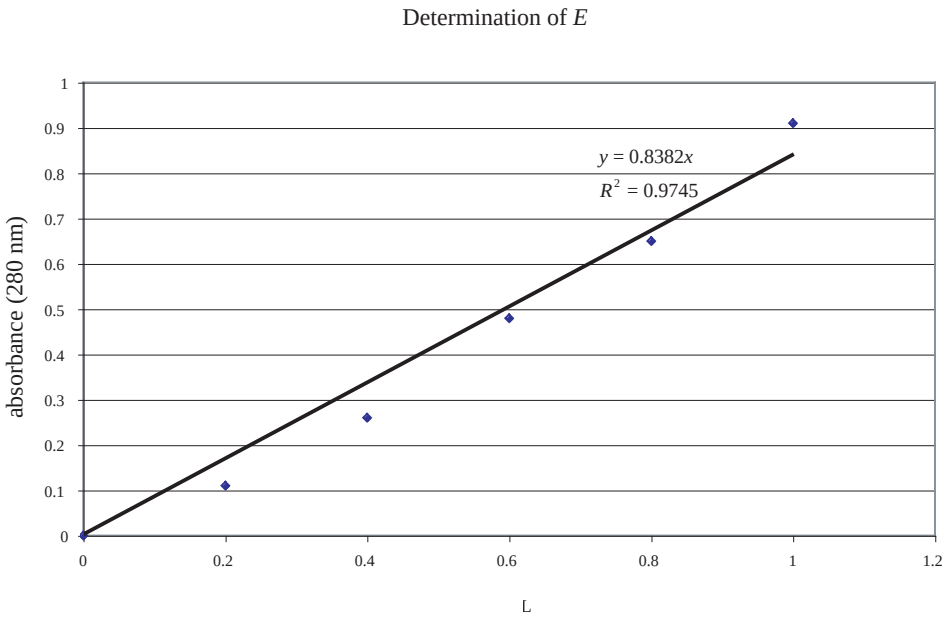

Figure 2

The six points were plotted in Figure 2 using Excel and linear regression was used. The value of E is the slope of the line. $E \approx 0.8382$.

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45. Spectrophotometry

50 μL of a 200 mg/mL stock solution of Actinomycin D was added to a batch of cell culture containing 5 mL medium, in order to stop RNA synthesis in the cultured cells. However, when the cells were examined following this treatment, there was evidence that RNA synthesis was still taking place, which suggested that an insufficient amount of Actinomycin D had been used. The scientist verified his entry in the laboratory notebook and determined that he had indeed added the right volume of Actinomycin D stock solution to the culture in order to obtain a final concentration of Actinomycin D of 2 mg/mL. Hence he decided to verify the concentration of Actinomycin D in the stock solution to determine if there was an error in the preparation of the stock solution. To do this, he first diluted the stock solution 50-fold, and then measured the absorbance of the diluted stock solution using a spectrophotometer set at a wavelength of 441 nm and obtained a reading of 0.7 absorbance units.

Actinomycin D has a molar extinction coefficient of 21,900 and a molecular weight of 1255. Use the Beer-Lambert equation for spectrophotometry, to determine the actual concentration of the stock solution in mg/mL.

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Solution:
The Beer-Lambert equation states that $A_\lambda = \lambda bC$, where A_λ is the sample's Absorbance value at a specific wavelength (or frequency), λ is the molar absorptivity coefficient of the material at that wavelength, expressed as L/moles-cm, b is the path length through the sample (usually 1 cm) and C is the concentration of the substance in moles/L. The absorptivity coefficient for every material is different, but for a given compound at a selected wavelength, this value is a constant. Since the units of λ, b and C cancel each other, A does not have units.

The molecular weight of a compound is the weight in grams of one mole of the compound. A mole of any compound contains 6.02×10^{23} molecules of the compound. In chemistry, 'M' (read as molar) denotes the concentration of a substance in a solution in moles/liter, or its molarity. A 1 molar solution of a compound, by definition, contains 1 mole of the compound in 1 L of solution.

Using the Beer-Lambert equation, we can first determine the molar concentration of the 50-fold diluted Actinomycin D solution as follows:

$$\begin{aligned} A_\lambda &= \lambda bC \\ 0.7 &= 21,900 \text{ L/moles-cm} \times 1 \text{ cm} \times ? \text{ moles/L} \\ ? \text{ moles/L} &= 0.7 \div 21,900 = 3.19 \times 10^{-5} \text{ moles/L} \\ 1 \text{ mole/L of Actinomycin D} &= 1255 \text{ g/L} \\ \text{Therefore, } 3.19 \times 10^{-5} \text{ moles/L} &= 0.04 \text{ g/L} \\ 0.04 \text{ g/L} &= .04 \text{ mg/mL} \end{aligned}$$

Since the original stock was diluted 50-fold for the spectrophotometric analysis, the actual concentration of the stock solution is,

$$0.04 \text{ mg/mL} \times 50 = 2 \text{ mg/mL}$$

Since the stock solution was supposed to have a concentration of 200 mg/mL, but was only 2 mg/mL, the amount of Actinomycin D used in the experiment was only 1/100th of the intended concentration. This would explain why there was no effect of the drug.

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Part XI

Absorbtion

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46. Absorption of Environmental Pollutants by Organisms

The pesticide DDT has estrogenic effects. Although DDT is no longer used in the U.S., it is still widely distributed worldwide. Humans have been shown to accumulate as much as $4\,\mu$g/g of body weight of DDT in their tissues.

If a man weighs 200 pounds, how much DDT might his body contain? (To simplify the situation, assume that all tissues in his body accumulate the same maximum amount of DDT. This is probably not correct.)

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Solution:
Let's use the unit canceling method to solve the second part of the problem. Conversion factors can be strung together into one long equation. First, it is necessary to convert the man's weight from pounds to grams. Then, a factor must be included to account for the fact that the man accumulated $4\,\mu$g/g of DDT in all his tissues. Finally It would be helpful to covert the answer from μg to grams. The resulting single equation is:

$$200\,\text{lb} \times \frac{454\,\text{g}}{1\,\text{lb}} \times \frac{4\,\mu\text{g}}{1\,\text{g}} \times \frac{1\,\text{g}}{10^{-6}\,\mu\text{g}} = 0.3632\,\text{g}$$

The accumulated DDT is 0.3632 g.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

47. Absorption of Environmental Pollutants by Organisms

Atrazine is a popular weed killer that strips male frogs of a key hormone and turns some frogs into hermaphrodites. Researchers exposed tadpoles to concentrations of atrazine ranging from 0.01 to 200 ppb (parts per billion). At concentrations of 0.1 ppb or above, 16–20% of the males developed extra testes, or even ovaries.

(a) Suppose that tadpoles are placed in an aquarium with 8 gallons of water and that 1 mL of water weighs one gram. How much atrazine (in grams) needs to be added to the aquarium to get a concentration of 0.1 ppb?

(b) If you have a digital scale that measures to the nearest mg, is it possible to weigh out this amount?

(c) What would you do to get a solution with the desired concentration that can be added to the solution?

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:

(a) Again, we use the unit canceling method.

$$8\,\cancel{\text{gallon}} \times \frac{3.785\,\cancel{\text{L}}}{1\,\cancel{\text{gallon}}} \times \frac{1000\,\cancel{\text{mL}}}{1\,\cancel{\text{L}}} \times \frac{1\,\cancel{\text{g}}}{1\,\cancel{\text{mL}}} \times \frac{0.1\,\text{g}}{10^9\,\cancel{\text{g}}} = 3.028 \times 10^{-6}\,\text{g}$$

So, 3.028×10^{-6} g of atrazine needs to be added to the aquarium to get a concentration of 0.1 ppb.

(b) This is too little to weigh out with a conventional balance.

(c) The accepted way to make a solution like this would be to make a more concentrated solution and dilute it, possibly through one or two serial dilutions.

Note that 0.1 ppb of atrazine is an extremely low concentration and yet seems to have an impact on wildlife.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

48. Absorption of Environmental Pollutants by Organisms

Phthalates are compounds that make plastics flexible and are used in many products including food wrap. Studies suggest that phthalates may activate receptors for estrogen, the primary female sex hormone. There is speculation that exposure to such estrogenic compounds may increase breast cancer incidence in women, reduce fertility in men, and adversely affect wildlife. Another study suggests that there is a link between phthalate exposure and premature breast development in girls as young as six months old.

One study suggested that margarine may pick up as much as 45 mg/kg of phthalates from plastic wrap. If you use a 1/4 pound of margarine that has absorbed phthalates at the rate of 45 mg/kg to bake a pizza crust, how much phthalate will half the pizza contain?

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Solution:
Here is one step-by-step strategy that begins by converting values into the metric system. First, convert 0.25 lb of margarine to kg:

$$\frac{1\,\text{lb}}{0.454\,\text{kg}} = \frac{0.25\,\text{lb}}{?}$$
$$? = 0.1135\,\text{kg}$$

So, weight of 0.25 lb margarine is 0.1135 kg units.

Margarine can absorb 45 mg of phthalates per kg so 0.1135 kg of margarine can take up:

$$\frac{45\,\text{mg}}{1\,\text{kg}} = \frac{?}{0.1135\,\text{kg}}$$
$$? = 5.1075\,\text{mg}$$

Thus, 0.1135 kg of margarine can absorb 5.1075 mg of phthalates.

There are therefore potentially 5.1075 mg of phthalates in the pizza, so one half the pizza might contain up to $2.55375\,\text{mg} \approx 2.55\,\text{mg}$ of phthalates.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

49. Absorbance and Transmittance in a Spectrophotometer

Transmittance, $\boldsymbol{T}$, is the intensity of the transmitted light and may be expressed as a percent, $\%T$, which is defined as

$$\begin{aligned}\%T &= \left(\frac{\text{Intensity of light through the sample in solvent}}{\text{Intensity of light through the pure solvent}}\right) \times 100\% \\ &= \left(\frac{I}{I_0}\right) \times 100\%\end{aligned}$$

Absorbance, $\boldsymbol{A}$, is used for graphical analysis because the absorbance is directly proportional to concentration, and is defined as

$$A = -\log\left(\frac{I}{I_0}\right) = -\log T$$

A **spectrophotometer** is being used to determine the concentration of a protein, cytochrome c, in sample solutions. A particular solution contains 5 mg/mL of cytochrome c. The solution transmits 40% of incident light relative to the pure solvent. **(a)** What is the absorbance in this case? **(b)** What is the predicted absorbance of a solution containing 15 mg/mL of the same substance (assuming that this concentration is in the linear range of the assay)? **(c)** What is the predicted transmittance of a solution containing 15 mg/mL of the same substance?

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:

(a) We are given $\%T = 40\%$, so, converting this to a decimal we see that $T = 0.40$. The absorbance of 5 mg/mL of this substance is $A = -\log T = -\log 0.40 \approx -(-0.398) = 0.398$.

(b) The relationship between absorbance and concentration is linear (within a certain range). Therefore, assuming linearity, this can be solved as a proportion problem:

$$\frac{5\,\text{mg/mL}}{0.398} = \frac{15\,\text{mg/mL}}{?}$$
$$? = 1.194$$

The predicted absorbance of a solution containing 15 mg/mL of cytochrome c is 1.194.

(c) Since $A = -\log T$, then $T = 10^{-A}$. From (b), we have $A = 1.194$ for 15 mg/mL of cytochrome c. Thus $T = 10^{-1.194} \approx 0.064 = 6.4\%$.

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

50. Absorbance and Transmittance in a Spectrophotometer

Absorbance, $\boldsymbol{A}$, is a measure of light absorption by a substance. Table 1 shows the absorbance for different concentration of a substance.

Table 1

Concentration mg/mL	0	1	2	3.5	4.8	6	7.3	8.4	9	9.4
Absorbance	0	0.08	0.18	0.31	0.41	0.52	0.64	0.75	0.79	0.83

(a) Graph that data from Table 1 with the concentration on the horizontal axis.

(b) Describe the shape of the curve in your graph in part **(a)**.

(c) Select two points that seem to best fall on a line that fits this data. Write an equation for the line that passes through those two points.

(d) Use your equation from part **(c)** and predict the absorbance for a concentration of 6.75 mg/mL.

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Solution:

(a) The data are graphed in Figure 1.

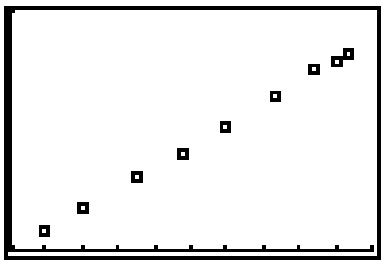

Figure 1

(b) The points seem to lie along a straight line.

(c) $A = 0.875C$, where A is the absorbance and C is the concentration in mg/mL.

(d) about 0.59

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51. Absorbance and Transmittance in a Spectrophotometer

Using spectrophotometry to determine the concentration of an analyte, Logarithms and antilogarithms, linear vs. nonlinear relationshipsLisa Seidman

A spectrophotometer has a light source that produces light, called the incident light and sends it to the sample cell with an intensity of I. A detector then measures the intensity of the light transmitted through the sample. If the intensity of the incident light is I_0 and the solution absorbs light, then the intensity of the transmitted light, I, is less than I_0. **Transmittance**, $\boldsymbol{T}$, is the intensity of the transmitted light and may be expressed as a percent, $\%T$, which is defined as

$$\begin{aligned} \%T &= \left(\frac{\text{Intensity of light through the sample in solvent}}{\text{Intensity of light through the pure solvent}} \right) \times 100\% \\ &= \left(\frac{I}{I_0} \right) \times 100\% \end{aligned}$$

Table 1 shows the % transmittance for different concentration of a substance.

Table 1

Concentration mg/mL	0	1	2	3.5	4.8	6	7.3	8.4	9	9.4
% Transmittance	100	82	67	49	38	30	23	18	16	15

(a) Graph that data from Table 1 with the concentration on the horizontal axis.

(b) Describe the shape of the curve in your graph in part **(a)**.

(c) Graph the data from Table 1 on the sheet of semi-logarithm paper on the next page. Use the horizontal axis for the concentration.

(d) Describe the shape of the curve in your graph in part **(c)**.

(e) Table 2 shows the %transmittance for different concentration of a substance.The first two lines of the table are the same as those in Table 1. Complete the third line of the table by calculating $-\log(\%$ Transmittance) for the % Transmittance values in the second line.

(f) Graph the first and third rows of Table 2 on "regular" rectangular graph paper.

(g) Describe the shape of the curve in your graph in part **(f)**.

(h) Describe how your graphs in part **(c)** and part **(f)** are alike and how they are different.

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Table 2

Concentration mg/mL	0	1	2	3.5	4.8	6	7.3	8.4	9	9.4
% Transmittance	100	82	67	49	38	30	23	18	16	15
$-\log$(% Transmittance)										

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:
(a) The data are graphed in Figure 2.

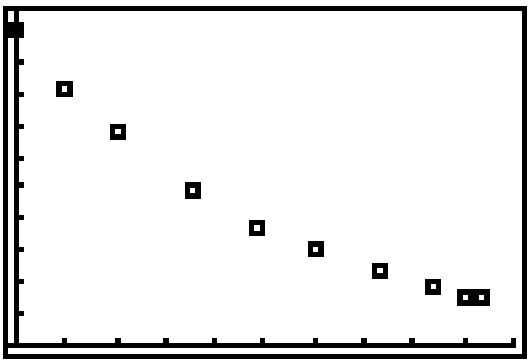

Figure 1

(b) The points do not lie along a straight line. The curve seems to be exponential.

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52. Protein estimation

In order to estimate the amount of protein in a given solution (solution X), a standard curve was generated by taking readings of light absorption for a set of standard solutions of known protein concentrations, using an instrument called the spectrophotometer. Light absorption by solution X was also measured, following a 50-fold dilution. The instrument response to protein concentration is linear up to a limit. Using the data provided, generate a linear graph by plotting the protein concentrations of the standard solutions against the corresponding instrument reading. Using this graph, estimate the protein concentration of solution X prior to dilution.

Protein Concentration of Standards (mg/mL)	Instrument Reading (Absorbance units)
0.10 mg/mL	0.14
0.25 mg/mL	0.35
0.50 mg/mL	0.70
0.75 mg/mL	1.05
1.00 mg/mL	1.40
1.25 mg/mL	1.52

Instrument reading for Solution X, following a 50-fold dilution = 0.91.

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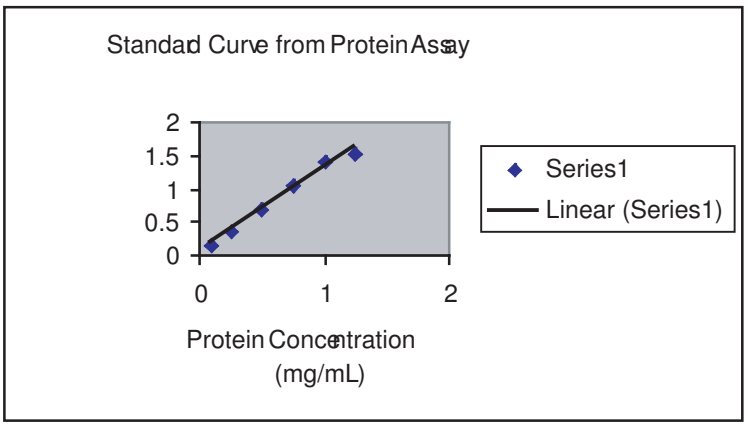
Solution:

The two variables in the problem are protein concentration and instrument response. Since the protein concentration is the independent variable, this should be plotted on the X or horizontal axis, and the instrument response, which is the dependent variable, on the Y or vertical axis. Plot the data provided on the graph paper, and connect the points with a straight line. Since the instrument response to protein concentration is linear only up to a limit, the last data point may not fall on the straight line. This line is the standard curve, and it is called the line of best fit. Now draw a horizontal line from 0.91 on the Y axis to the line of best fit, and drop a perpendicular to determine the corresponding protein concentration on the X axis. This is the protein concentration of the diluted solution X. Since the original solution was diluted 50-fold, multiplying this protein concentration by 50 will give you the protein concentration of the original solution X.



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Part XII

Quality Control

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53. HIV Quantification

HIV antibody detection commonly uses an ELISA (Enzyme Linked Immunosorbent Assay) method. Positive tests are indicated by a color change. The intensity of the color can be used to quantify the antibodies. This is done by using OD (optical density) readings at 450 nm.

The ELISA protocol is followed carefully to test the serum of 10 individuals. There are three controls: positive, negative, and assay without serum. You are given the following:

1. The lowest positive value is 0.500
2. Values between 0.300 and 0.499 are questionable and require further testing.
3. Values below 0.300 are negative

How would you interpret the following ELISA optical densities for individuals being tested?

Individual or Control	**OD 450**	**Result**
Positive Control	1.732	
Negative Control	0.124	
Assay Control (no serum)	0.101	
Individual 1	1.846	
Individual 2	0.103	
Individual 3	0.362	
Individual 4	0.401	
Individual 5	0.583	
Individual 6	0.855	
Individual 7	1.632	
Individual 8	1.951	
Individual 9	0.122	
Individual 10	0.490	

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Solution:

Individual or Control	OD 450	Result
Positive Control	1.732	Positive
Negative Control	0.124	Negative
Assay Control (no serum)	0.101	Negative
Individual 1	1.846	Positive
Individual 2	0.103	Negative
Individual 3	0.362	Questionable
Individual 4	0.401	Questionable
Individual 5	0.583	Positive
Individual 6	0.855	Positive
Individual 7	1.632	Positive
Individual 8	1.951	Positive
Individual 9	0.122	Negative
Individual 10	0.490	Questionable

If the positive control showed a reading of 0.250 at OD 450 nm, the remaining entire set of tests would be questionable and would need to be repeated with an appropriate positive control.

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54. Statistical Process Control on a Fermentation Reactor

A large industrial fermentation reaction takes approximately three days to reach completion. During that time, optimum temperature and pH conditions must be maintained. In order to do so, operators monitor the pH, which is controlled by the automatic addition of sodium hydroxide, and the effect of the cooling water, which circulates through large coils inside the fermentation reactor. Statistical process control methods are used to determine if a procedure remains "under control." If a procedure is under control, no action needs to be taken by the operators.

Procedures

(a) From the following chart, determine the number of data points in Table 1.

(b) What are the lowest and highest data points.

(c) Determine the range of data, that is, the distance between the lowest and highest data points.

Table 1: Hourly Temperature Readings from the Fermentation Reactor

Seq./Date	1	2	3	4	5	6	7	8	9	10	11	12
July 6	36.8	36.2	36.8	37.2	35.0	37.6	35.9	36.9	36.2	37.0	36.7	34.4
July 7	36.0	37.2	36.8	35.9	36.9	35.5	35.8	36.0	36.9	35.8	36.6	36.6
July 8	36.6	35.5	36.0	36.5	36.3	35.7	36.6	36.8	36.7	35.8	37.4	37.4
	13	14	15	16	17	18	19	20	21	22	23	24
July 6	37.1	36.3	36.2	36.5	36.9	36.2	37.0	34.0	36.2	37.4	35.9	36.8
July 7	36.8	36.2	35.5	36.7	36.7	36.4	34.5	36.5	36.0	36.4	36.7	37.1
July 8	36.6	34.4	37.3	36.0	36.9	35.5	38.1	35.5	36.8	35.1	36.7	36.2

(a) Construct a histogram to represent the data.

(b) Explain why you selected this width for the intervals in the histogram or why you selected this number of intervals.

(c) Determine the mean (average).

(d) Determine the standard deviation.

(e) Plot the Fermentation Reactor data as it would be described by a statistical process control chart including:

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i. An x-axis showing the hourly time intervals

ii. An appropriate range on the y-axis for the measured temperature in Celsius degrees.

iii. A horizontal line showing the mean, $\overline{x}$.

iv. Horizontal lines showing $\overline{x} \pm 1\sigma$.

v. Horizontal lines showing $\overline{x} \pm 2\sigma$.

vi. Horizontal lines showing $\overline{x} \pm 3\sigma$.

The remaining questions concern interpretation of the SPC graph. This will allow you to determine if the process is under control.

(a) At any time during the run were there seven consecutive values more than one sigma above or below the mean?

(b) At any time during the run were there three or three out of four consecutive results outside either plus one sigma or minus one sigma?

(c) At any time during the run were there two or two out of three consecutive results outside either plus two sigma or minus two sigma?

(d) At any time during the run was there a value beyond plus three sigma or minus three sigma?

(e) At any time during the run was there a change from one value to the next that crossed four sigma lines?

(f) Was there any time during this production run when the operator(s) should have made an adjustment to the temperature control?

(g) Is there evidence that the operator made any adjustments to the temperature control?

(h) If the desired mean temperature is 37°C (human body temperature), are there adjustments that the operator(s) can make?

(i) If the desired standard deviation is 0.5°C, are there adjustments that the operator(s) can make?

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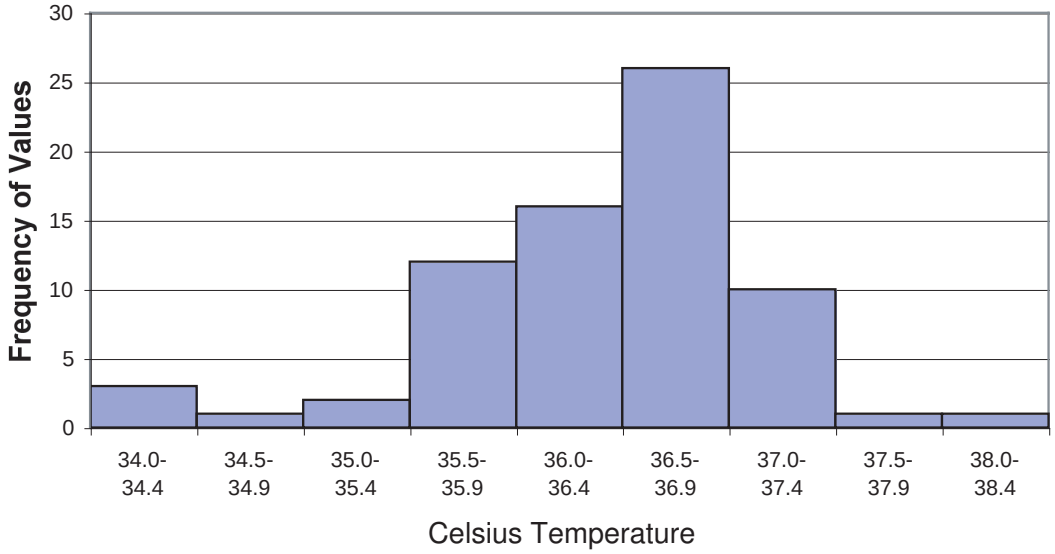
Solution:

(a) There are 72 data pounts.

(b) The highest point is 38.1 and the lowest is 34.

(c) The highest point is 38.1 and the lowest is 34. Thus, the difference is 4.1.

(d) Histograms may vary. One possible histogram is shown below.

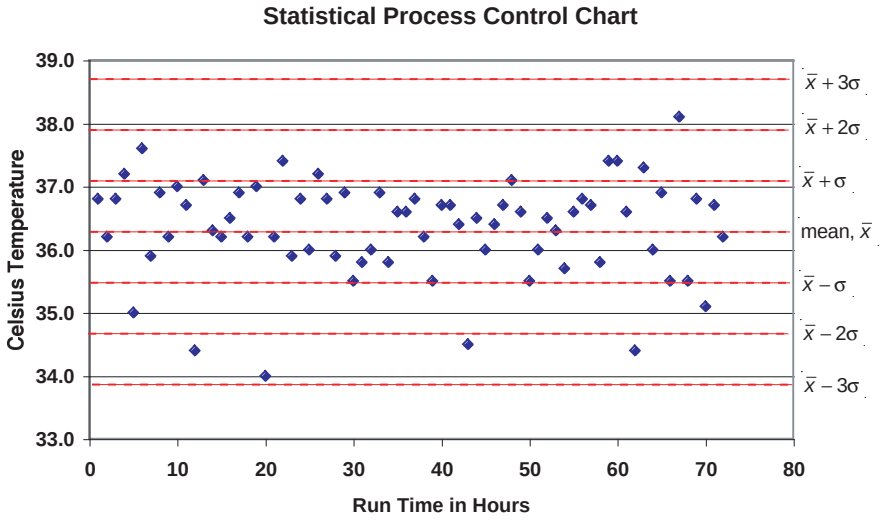


(e) Answers may vary.

(f) The mean is about 36.35.

(g) The standard deviation is about 0.77.

(h) The graph below is the statistical process control chart.



(i) No

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(j) No

(k) No

(l) No

(m) No

(n) No

(o) No

(p) Yes, the operator(s) may increase the temperature by increasing the amount of heat energy supplied to the reaction or, if the reaction is exothermic, the operator(s) may increase the temperature by reducing the amount of heat being removed by cooling water.

(q) No, in order reduce the variability in the process; equipment or system changes must be made.

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55. The Use of Standard Deviation to Analyze Laboratory Data

Many activities or experiments performed in the lab or in the field generate large amounts of data that must be analyzed and interpreted by the technician to determine if the data fit into the framework of the system being studied. As a technician you will be required to use statistical analysis to make decision about the nature of the data collected.

Data has been generated that relate to the level of bacterial concentration in rain fall.

Thirty successive 100 mL rainwater samples were collected in sterile vessels during thirty successive rainstorms to determine the concentration of bacteria typically found in falling rainwater. Records indicate that samples #21 through #25 were taken during and immediately after a hurricane passed through the collection site. Each 100 mL sample was filtered through a Millipore membrane filter (0.22 μm pore size) and the filter disks were placed on Tryptic Soy Agar media. After 36 to 48 hours incubation at 37°C, the number of bacterial colonies were counted and reported as Colony Forming Units (CFU) per 100 mL.

(a) Calculate the mean and the standard deviation of the six samples from the data set in Table 1.

(b) Recalculate the mean and the standard deviation of the samples in the data, leaving out samples #21, #22, #23 and #24.

(c) Calcuate the mean and standard deviation of samples #21, #22, #23 and #24 only.

(d) What is it about the circumstances of the sample collection and the experiment that make it appropriate to isolate samples #21, #22, #23 and #24 from the rest of the data set?

Table 1

Sample #	CFU per 100 mL	Sample #	CFU per 100 mL	Sample #	CFU per 100 mL
1	27	11	45	21	2.4×10^3
2	35	12	56	22	4.3×10^3
3	22	13	102	23	45.2×10^3
4	30	14	46	24	7.2×10^3
5	45	15	38	25	99
6	35	16	68	26	45
7	67	17	55	27	78
8	21	18	64	28	56
9	78	19	39	29	34
10	98	20	46	30	52

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:

(a) First, calculate the mean:

$$\begin{aligned}\overline{x} &= \frac{\text{sum of the items in the data set}}{\text{number of items in the data set}} \\ &= \frac{\sum x}{n} \\ &= \frac{27 + 35 + 22 + \cdots + 56 + 34 + 52}{30} \\ &= \frac{60{,}481}{30} \\ &\approx 2016.03\end{aligned}$$

The mean is about 2016 CFU per 100 mL of rainwater.

Next, determine the standard deviation:

$$\begin{aligned}s &= \frac{\sum(x - \overline{x})^2}{n - 1} \\ &= \frac{(27 - 2016.03)^2 + (35 - 2016.03)^2 + \cdots + (34 - 2016.03)^2 + (52 - 2016.03)^2}{30 - 1} \\ &\approx 8{,}298.91\end{aligned}$$

The standard deviation is about 8,298.91 CFU per 100 mL of rainwater.

(b) After omitting samples #21, #22, #23 and #24 from the data, the mean and standard deviation of the remaining 26 samples are $\overline{x} \approx 53.12$ and $s \approx 23.04$ CFU per 100 mL of rainwater.

(c) The mean and standard deviation of samples #21, #22, #23 and #24 are $\overline{x} \approx 14{,}775$ and $s \approx 20{,}379.14$ CFU per 100 mL of rainwater.

(d) Reviewing the size of the standard deviation in each computation makes it clear that there is a significant difference imposed on the calculation when the #21 through #24 samples are either included in the data set or, even, isolated from the rest of the data set. While it is never permitted in scientific investigation to simply discard data that seems to be outside the domain of the main data points, it is acceptable to isolate and account for extreme differences in the parameters that guide the overall experimental protocol. In this case, the four data points in question were collected under extreme conditions that did not exist during the collection of the remaining 26 data points, specficially, the activity of rain fall associated with a hurricane. Since experimental protocols attempt to maintain as many parameters within defined limits as possible, it is acceptable to recognize the vast difference in weather conditions that would likely cause such an extreme difference in sample results, allowing us to isolate and describe these data points as occurring under very different conditions. In fact, these data points might provide a jumping off point for a new series of investigations relating to the effect of extreme weather on bacterial loads in precipitation.

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56. Using Standard Deviation to Describe the Repeatability of an HIV Assay

A contract testing lab that serves the biotechnology industry wants to market a new, rapid assay for detecting HIV viral DNA in drug products. In order to perfect the assay, the company's R&D scientists intentionally add known amounts of viral DNA to suspensions of products. They then test whether their assay gives consistent results. They add three levels of viral DNA to product suspensions and repeat each level five times. They use standard deviation (SD) and relative standard deviation (RSD) as measures of the repeatability (consistency) of the test. The scientists ultimately want to be able to guarantee their customers that they will be able quantify HIV DNA in samples with better than $\pm 0.040\,\mu$g SD and $\pm 4.00\%$RSD. Have they achieved their goal?

Observe that the more repeatable the values, the lower (better) the value for standard deviation.

Round the answers to match with the specifications, after all calculations are completed.

$$\text{SD} = \sqrt{\frac{\sum (x - \overline{x})^2}{n - 1}}$$

$$\text{RSD} = \frac{\text{Standard deviation} \times 100\%}{\text{mean}}$$

Amount of DNA added to suspensions (μg)	Amount of DNA detected by assay (μg)	Average (μg)	SD (μg) Round to number of places in the specification	RSD Round to number of places in the specification
1.00	1.02, 0.99, 1.03, 0.96, 1.01			
10.00	9.87, 10.16, 9.18, 10.13, 9.89			
25.00	24.45, 23.25, 24.04, 24.65, 24.32			

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

Solution:

Amount of DNA added to suspensions (μg)	Amount of DNA detected by assay (μg)	Average (μg)	SD (μg) Round to number of places in the specification	RSD Round to number of places in the specification
1.00	1.02, 0.99, 1.03, 0.96, 1.01	1.002	$0.02775 \approx 0.028$	$2.76935 \approx 2.77\%$
10.00	9.87, 10.16, 9.18, 10.13, 9.89	9.846	$0.39539 \approx 0.395$	$4.01570 \approx 4.02\%$
25.00	24.45, 23.25, 24.04, 24.65, 24.32	24.142	$0.54559 \approx 0.546$	$2.25993 \approx 2.26\%$

They have not achieved their goal. The standard deviation is too high when 10 and 25 micrograms of DNA were assayed. The RSD is too high at the 10 microgram level. Further refinements to their technique are required.

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57. Descriptive Statistics (Histograms) in a Production Setting

A company is manufacturing restriction enzymes for use in biotechnology research laboratories. They test the activity of 45 batches of enzyme and find the results below.

(a) Organize these data into a frequency table with between five and ten classes.

(b) Display these data in a histogram. Explain the results in words.

DATA: (All values are in international units (IU) of activity per mg protein):

3450	4589	3563	4378	3489	4832	2987	3467	3567	4376
5621	2327	4637	3098	3367	5711	4356	3876	3451	5100
4132	3298	3786	4130	4298	3785	2900	3456	3980	4521
4782	3671	3961	4328	4381	3481	3762	3091	3726	4561
3981	3019	3281	2654	3456					

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Solution:

(a) These data can be summarized using eight classes as shown in the following table.

RECORDED ACTIVITY	FREQUENCY
2,000–2,499	1
2,500–2,999	3
3,000–3,499	13
3,500–3,999	11
4,000–4,499	8
4,500–4,999	6
5,000–5,499	1
5,500–5,999	2

(b) A histogram of the data is shown in Figure 1.

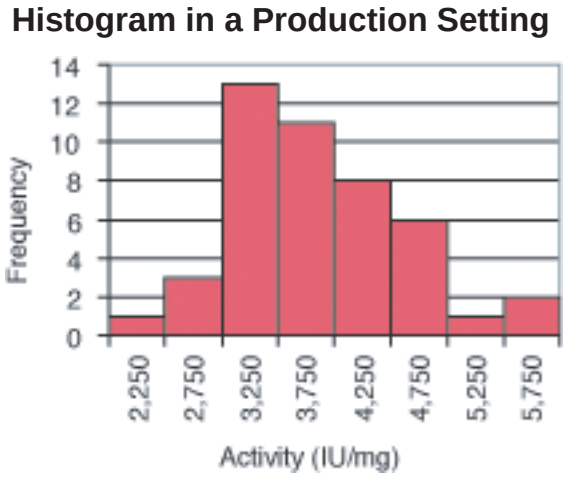

Figure 1

Most of the lots are of intermediate activity with a few batches that are of lower activity (between 2,000 and 3,000 IU/mg) or higher activity (5,000–6,000 IU/mg). These data appear roughly normal, although slightly skewed toward the higher activity.

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58. Relative Percent Error of An HIV Assay

A contract testing lab that serves the biotechnology industry wants to market a new, rapid assay for detecting HIV viral DNA in drug products. In order to perfect the assay, the company's R&D scientists intentionally add known amounts of viral DNA to suspensions of products. They then test whether their assay gives the expected results. They add five levels of viral DNA to product suspensions and repeat each level three times. The scientists ultimately want to be able to guarantee their customers that they will be able quantify HIV DNA in samples with relative percent error between -4.00% and 4.00%. Based on their data in Table 1, have they achieved their goal?

Use Relative Percent Error to evaluate their success.

$$\text{Relative percent error } = \frac{\text{true value } - \text{ average measured value}}{\text{true value}} \times 100\%$$

Round the percent error results to match the specification after all calculations are completed.

Observe that the more accurate their values, the lower (better) the value for percent error.

Table 1

Amount of DNA added to suspensions (μg)	Amount of DNA detected by assay (μg)	Average (μg)	Relative % error
1.00	0.91, 0.99, 1.05		
5.00	4.78, 6.34, 4.21		
10.00	9.89, 10.46, 8.78		
15.00	15.08, 15.92, 17.65		
25.00	22.04, 21.45, 24.32		

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Solution:

Amount of DNA added to suspensions (μg)	Amount of DNA detected by assay (μg)	Average (μg)	Relative % error
1.00	0.91, 0.99, 1.05	0.98	2.00
5.00	4.78, 6.34, 4.21	5.10	−2.20
10.00	9.89, 10.46, 8.78	9.71	2.90
15.00	15.08, 15.92, 17.65	16.22	−8.13
25.00	22.04, 21.45, 24.32	23.94	4.24

These data suggest that at the two highest concentrations they have not achieved the desired accuracy. Refinements in their technique are required.

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Part XIII

Multi Use

Work on this project was partially funded by the National Science Foundation. NSF Award #: DUE 00 03065

59. Determining the Protein Concentration of a Solution Containing a Protein, Lactate Dehydrogenase

Given the information in Table 1 found in the results section, determine the protein concentration (mg/mL) of the solution containing the protein lactate dehydrogenase (LDH). This protein, known as an enzyme, speeds up a reaction where lactate is converted to pyruvate. This enzyme is a common product sold by several biotechnology companies to be used in research. It is also bought and used as a standard in clinical laboratories since the presence of this protein in the circulatory system indicates heart muscle damage (i.e. a heart attack).

Procedure for the test: To do the test, the technician prepares a rack of tubes. **1.** One tube serves as a blank as it contains NO protein only the liquid used with the protein **2.** Several tubes containing increasing concentrations of the protein. **3.** Bovine serum albumin (BSA) which are used to prepare the calibration curve. **4.** Last there are several containing dilutions of the unknown containing LDH. To all of these tubes a constant amount of dye is added. The dye binds to the protein and as a result, changes color. This color absorbs light at some maximal wavelength, in this case 595 nm.

$$\text{Dye} + \text{protein} \rightarrow \text{dye protein complex (maximal absorbance at 595 nm (nanometers))}$$

Table 1

Tubes 1–5	Tubes 6–9	Tube 10
Increasing concentration of (BSA)	Different concentrations of the sample	Blank

The tubes are allowed to sit for the appropriate amount of time so that the maximum amount of dye that can bind to protein occurs. During this time, the technician turns on the spectrophotometer so that the light source can warm up. When the tubes are ready, the technician uses the blank "to zero" the machine. The machine stores this value so it can subtract that reading from the sample readings. Now the technician obtains the absorbance readings for all of the samples. From this information, which is given in results, the technician can determine the protein concentration of the sample which contains LDH.

Background Information on How the Test Works: (If you REALLY know what is happening then you should be able to answer the questions in the following section!)

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The more protein present in the tube, the more dye binds to the protein resulting in a darker color in the tube. A technician measures the differences in color produced as a result of the differences in protein concentration in the tubesby using a spectrophotometer, a machine that measures light at selected wavelengths.

A constant light intensity at a selected wavelength is sent through the sample (I_0), the sample absorbs some of the light resulting in a decreased intensity (I) exiting the sample. The decrease in light intensity is measured by a detector (i.e. containing a photodiode) such that the percent of transmission $\%T = \frac{I}{I_0} \times 100$. The signal is converted to a digital read out.

- What is the color of the solution if there is 100% transmission?
- What is the color of the solution if there is 0% transmission?
- What is the color of the solution if there is 100% absorbance by the solution?
- What is the color of the solution if there is 0% absorbance by the solution?
- What is the relationship between absorbance and transmission?

If the spectrophotometer is set to display absorbance it will calculate this by:

$$\text{Absorbance } = -\log_{10}\left(\frac{I}{I_0}\right)$$

Note, if plotted, the relationship between the concentration of the material being measured in a sample and the transmittance of light through the sample is NOT linear. It is exponential. However there is a linear relationship between the absorbance of light by a sample and the concentration of material being measured. Therefore, measuring absorbance is used to determine concentration and not transmittance AND within a certain **range** of protein concentrations the binding of the dye to protein and thus the color is linear.

- Why is the relationship between concentration and transmittance NOT linear when the relationship between concentration and absorbance IS linear? Explain your reasoning.
- How can absorbance be used to determine the concentration of a protein-dye complex in a sample? Explain your reasoning.

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Table 2: Results of the Experiment (explanation for the table given below table)

Bovine Serum Albumin Protein Standards (mg/mL)	Absorbance readings at a wavelength of 595 nm	Protein Containing Solution volume (**dilution**)	Absorbance readings at a wavelength of 595nm	Determined protein concentration in mg/mL
1) 0.20 mg/mL	0.25	6) 100 μL	2.50, 2.40	
2) 0.40 mg/mL	0.37	7) 100 μL, (**1/5**)	0.40, 0.42	
3) 0.60 mg/mL	0.58	8) 100 μL (**1/50**)	0.04, 0.05	
4) 0.80 mg/mL	0.82	9) 100 μL (**1/100**)	0.02, 0.01	
5) 1.00 mg/mL	0.91	10) **100 μL blank**	0.0	0.0

Explanations for the Table

- The first column provides information on the concentration of the protein standards prepared from bovine (cow) serum albumin (BSA). Albumin is the protein of highest concentration in the blood and is collected from cows in slaughterhouses. Therefore it is a cheap protein that can be purchased from a variety of sources. Usually a 1.00 mg/mL solution is prepared and diluted appropriately to make the standards. One hundred microliters of standard will be used per tube.
- The second column provides readings obtained at 595nm for each tube of standard. In this case, do you think transmission was measured or was it absorbance? To make sure that the reading only reflects changes in protein concentration and not the solution or buffer that the protein is dissolved in, a blank is prepared using 100 μL of the solution (at the end of column three) and the absorption reading is set to zero at 595nm.
- The third column provides information for one sample of unknown protein concentration containing the protein, lactate dehydrogenase. Several dilutions of this sample, as indicated by the bolded fraction (1/5 means sample was diluted 1 : 5), were made so hopefully two of the absorbance readings obtained fall on the linear portion of the calibration curve. In each case the sample size was 100 μL.
- The fourth column provides information on the absorption readings obtained for the sample. Duplicates were run for each dilution.
- The fifth column is the one you need to fill out. However, based on the absorption readings, not all of this column will have answers.

Plotting the Data and Determining the Protein Concentration for the Sample

- First, determine which are the dependent and independent variables as this will determine which data should be plotted on the y and x axis.
- Second, determine a linear regression model on the data for the standards to produce what is known as the calibration curve. Even though you "zeroed the machine," and therefore artificially produce a zero point, DO NOT use a zero point on your plot, only the values that were generated for the calibration curve. It is preferred that you use measured data instead of artificially generated data to interpolate the data.

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- Third, from the calibration curve and the absorbance readings obtained for your sample, determine which absorbance readings lie on the calibration curve.
- Finally determine the protein concentration of the solution containing the protein, lactate dehydrogenase. Remember NOT to use any points that result in extrapolation of the data, only interpolate!

Questions:

(a) What is the linear range for the spectrophotometer used in this experiment? Explain your reasoning.

(b) Why is it important that the technician do a calibration curve in this test?

(c) Which values were retained and which points were thrown away in determining the calibration curve? Explain your reasoning.

(d) What absorbance values were retained and which ones were thrown away when determining the protein concentration of your sample? Explain your reasoning.

(e) Why do you think the technician used these dilutions for the sample? Do you agree with this choice? If you decided to do another test to determine protein concentration of the sample, what dilutions would you use and why?

(f) Besides determining protein concentration, can this type of test be used to determine if the spectrophotometer is working correctly, explain.

(g) Explain why you "zeroed the machine" and what this must include?

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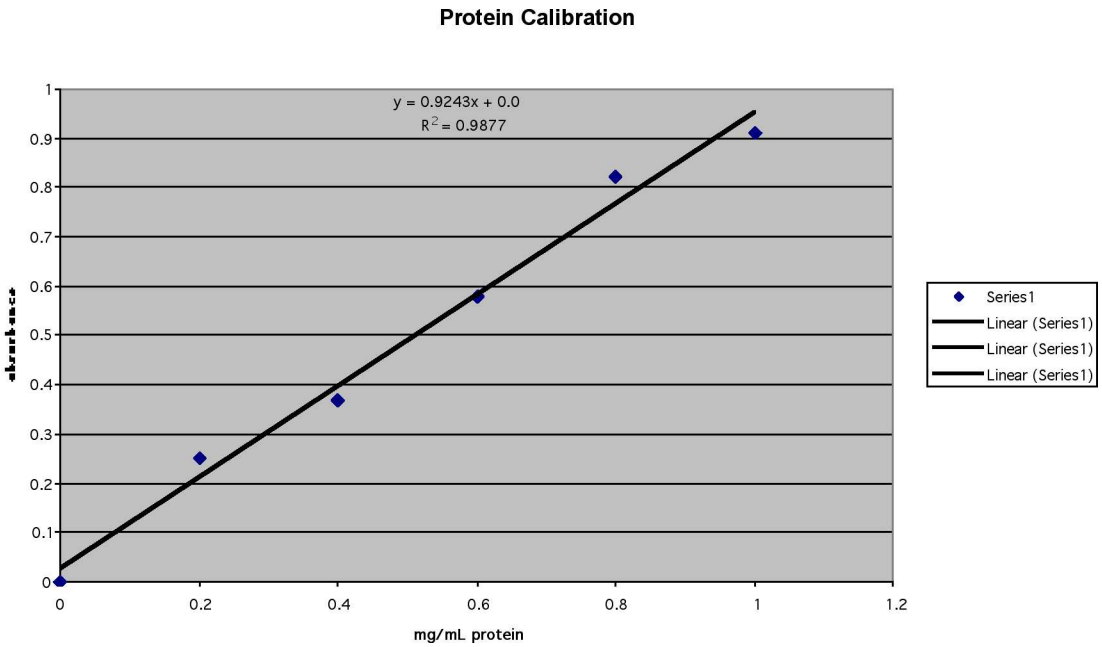

Determination of supernatant protein concentration: Using only the two readings obtained at around 0.3 A (only these readings are in the linear range of the assay), and averaging these values, $A = 0.41$, the concentration is calculated. Using the method of least squares, or the regression equation where $Y = slope(X) + Y$ intercept (which is 0 in this case): $0.41 = (slope)X$ so $X = \dfrac{0.41}{\text{slope}}$. As calculated by the program, slope = 0.9243. Thus, $y = 0.9243x + 0.0262$, $R^2 = 0.9877$, and $X = 0.44\,\text{mg}\times$ dilution factor (5) /0.1 mL$= 22.0\,\text{mg/mL}$.

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60. Optimizing an Enzyme Assay: Lactate Dehydrogenase

Technicians commonly have to devise and optimize enzyme assays. For example in this case, a technician is perfecting an assay that detects the activity of an enzyme known as lactate dehydrogenase. It is found in a variety of muscle cells. There are two forms of this enzyme otherwise known as isoenzymes. One type is found in heart muscle, and the other is found in skeletal muscle. In fact assaying for the presence of the heart isoenzyme in a person's blood has proven a quick way to determine if heart muscle has been damaged as the result of a heart attack. The concentration of the heart muscle associated LDH increases dramatically in the blood of patients who have had a heart attack. Commerical LDH is routinely used in clinical laboratories as a standard when a person's blood is being assayed for this enzyme.

In this experiment the technician has prepared a solution of the enzyme and is figuring out the optimum concentration to use in the enzyme assay. To determine the optimal amount of solution to use in the assay, the technician runs several assays varying the amounts of the solution. In the first sample, the technician uses 10 μL straight and in the rest of the samples, the technician dilutes the solution in buffer before using it. The sample concentration that produces the best linear response is selected. Dilutions that result in too low or too high of an activity and therefore are difficult to measure are not considered optimal enzyme concentrations. To do the assay, the technician puts the Master Mix into the tube in the spectrophotometer, adds the solution (source of enzyme), quickly mixes using a up down motion with a small plunger, and closes the lid of the spectrophotometer. He or she then quickly starts to monitor the reaction by recording the change in absorbance.

Part A: Preparation of a Master Mix: You are going to be assaying 8 samples, so you need to prepare a Master Mix, which some technicians refer to as the Cocktail. To make sure you have enough Cocktail, prepare for 9 tubes instead of 8. The total volume for each tube is 2.9 mL mix and 0. mL sample. The Master Mix is composed of 0.15 M CAP buffer, pH 10,

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6 mM NAD, and 0.15 M lactate. **You are given the following ingredients, show how you would prepare the Cocktail: (a)** A stock solution of 1.5 M CAP pH 10, **(b)** solid sodium lactate (molecular weight = 112 g/mole), and **(c)** a 5 mL vial of freshly reconstituted 10 mM NAD. You do not need to know what the ingredients in the assay to prepare the Cocktail, but for your information, CAP is a buffer that works well in the pH range of 9 to 11, lactate is a substrate for the enzyme as shown by the equation below, and NAD or nicotinamide adenine dinucleotide is a co-factor that is used by a class of enzymes known as dehydrogenases and it helps the enzyme to do its job In this case the protein being assayed is an enzyme known as lactate dehydrogenase and it catalyzes the reaction shown below.

Part B: Once the Cocktail is prepared you are ready to detect the protein, in this case the enzyme lactate dehydrogenase, which catalyzes the following reaction:

$$\text{L-lactate} + \text{NAD}^+ \longrightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+.$$

The reaction occurs in tube that is sitting in a machine known as a spectrophotometer. The spectrophotometer shines a beam of light through the tube at a specific wavelength. Depending what is in the sample, light is absorbed as the beam passes through it. For this assay, the wavelength is set to 340nm so that the formation of NADH can be measured. NADH, NOT NAD, shows maximal absorbance at this wavelength. As part of trying to figure out the best conditions for the assay, different concentrations of the enzyme are used starting with 10 μL straight enzyme, the 1:10 dilution of this solution and finally a 1:50 dilution. It is important to find a dilution that produces the best linear reaction rate that can be measured.

Using Excel or a similar program, plot the reactions, change in absorbance vs. time, and determine the rate of reaction at an absorbance of 340 nm from the data collected below for each assay. From your plots, determine which dilution gives the best results.

Time (sec)	10 μL straight (series 1)	1 : 10 dilution (series 2)	1 : 50 dilution (series 3)
15	0.056	0.05	0.002
30	1.2	0.12	0.004
45	1.5	0.19	0.006
60	2	0.26	0.008
75	2.5	0.37	0.010
90	2.5	0.42	0.012
105		0.47	0.014
120		0.52	0.016
135			0.021
150			0.032

Part C: Determination of Specific Activity: Once you have determined the best dilution to run the assay, you are ready to figure out its specific activity. Specific activity is defined as the measured activity of the enzyme per a measurement of amount for example mg. Depending on the enzyme, the activity can be expressed in a variety of formats. For lactate dehydrogenase, and a number of other enzymes, activity is expressed in units, thus specific activity is equal to units/mg. In this example, units is equal to the change in absorbance/min/6220 M^{-1}) $\times$ 10^6 μM/M $\times$ total volume of reaction in liters. The number 6220 M^{-1} is the extinction coefficient for NADH at 340 nm. Therefore a unit is equal to umoles of NADH formed per minute.

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In another assay, the technician determines protein concentration, and it is determined that the protein concentration of the solution containing the enzyme is 22 mg/mL Showing all work, calculate the specific activity for this enzyme.

Part D: Answer the following questions

1. From the graph where you plot change in absorbance vs time, what dilution is optimal for producing a measurable linear rate?
2. What is the linear range for the spectrophotometer being used in this experiment?

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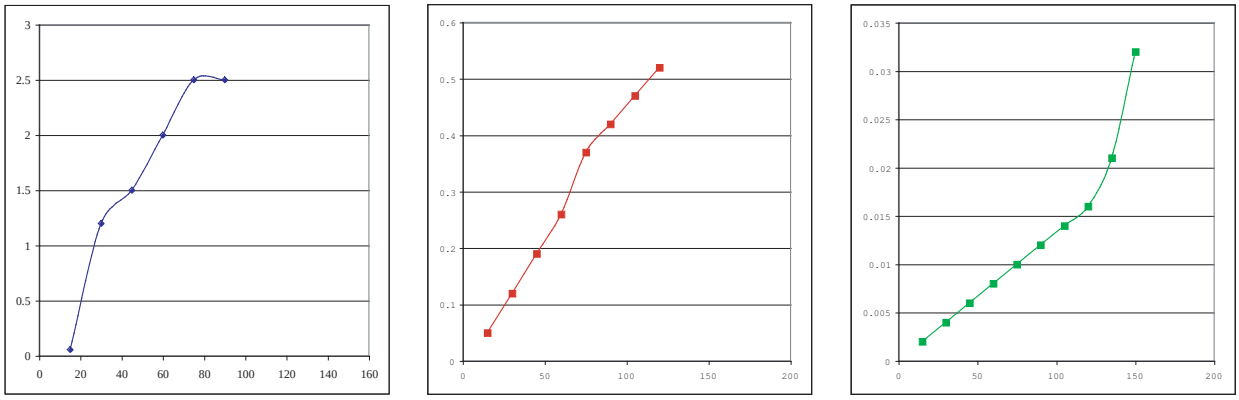
Solution:

Part A: Preparation of Master Mix: Nine tubes means that you are preparing for $(9 \times 3\,\text{mL}) = 27\,\text{mL}$ total reaction so the concentration of the reactants need to be added accordingly. Using the formula $V_1C_1 = V_2C_2$ we have $(V_1)(1.5\,\text{M}) = (0.027L)(0.15\,\text{M})$ the amount of CAP stock solution to add is 2.7 mL. The amount of solid sodium lactate is 0.15 moles/liter $\times 0.027\,\text{L} \times 112\,\text{g/mole} = 0.45\,\text{g}$. For NAD, $(V_1)(10\,\text{mM}) = (0.027\,\text{L})(6\,\text{mM})$, we find that $V_1 = 0.0162\,\text{L}$ or 16.2 mL, bring the volume up to $(9 \times 2.9\,\text{mL}) = 26.1\,\text{mL}$.

Part B: The graphs of the three series are in the figure below.



(a) Series 1 (b) Series 2 (c) Series 3

Part C: Determination of Specific Activity: Specific activity is equal to units/mg. The concentration of the supernatant is 22 mg/mL. One choice is series 2 or the 1 : 10 dilution. Therefore the concentration of supernatant is $0.010\,\text{mL} \times 22\,\text{mg/mL}/10 = 0.022\,\text{mg}$. The change in absorbance per minute is $(0.26/\text{min}/6220\ \text{M}^{-1}) \times 10^6\,\mu\text{M/M} \times 0.003\,\text{L} = 0.125\,\text{U}/0.022\ \text{mg} = 5.7\text{U/mg}$.

Part D:

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Workshop Participant Names and Addresses

Biotechnology

Zoe A. Fitzgerald
Professor of Science/Dept. Chair
Saint Charles Community College
4601 Mid Rivers Mall Drive
Saint Peters, Mo 63376
Telephone: (636) 922-8578
Fax: (636) 922-8478
E-mail zfitzgerald@stchas.edu

Linnea Fletcher
Biotechnology Program Coordinator
Austin Community College/Rio Grande Campus
1212 Rio Grande
Austin, TX 78701
(512) 223-3284
linneaf@austincc.edu

Samuel Gaskins
Associate Professor
Guilford Technical Community College
P O Box 309
Jamestown NC 27282
(336) 334-4822
gaskinss@gtcc.cc.nc.us

Elaine Johnson
Bio-Link Director
ATE Center
City College of San Francisco
1855 Folsom Street, Suite 643
San Francisco CA 94103
(415) 487-2472
ejohnson@biolink.ucsf.edu

Kunthavi Natarajan
Associate Professor
Biotech. Program, Biology Dept.
Sinclair Community College
Dayton, OH 45402
Ph: (937) 512-4579
Fax: (937) 512-3057
Kunthavi.Natarajan@sinclair.edu

Sandra Porter
Senior Scientist
Geospiza, Inc.
2442 NW Market St., PMB #344
Seattle WA 98107
(206) 633-4403
sandy@geospiza.com

Meg Rawls
Biology Instructor
Carteret Community College
3505 Arendell Street
Morehead City, NC 28557
(252) 222-6383
mfr@carteret.edu

Lisa Seidman
Program Director Giotechnology Laboratory
Madison Area Technical College
3550 Anderson Street
Madison, WI 53704
(608) 246-6204
lseidman@matcmadison.edu

William H. Woodruff
Dept Head of Biotechnology
Alamance Community College
P O Box 8000
Graham NC 27253
(336) 506-4224
woodruff@alamance.cc.nc.us

Mathematics

Elisabeth Gambler
Professor of Mathematics
Vermont Technical College
54 Pine Ridge Drive
Randolph Center VT 05061
(802) 728-1205
egambler@vtc.edu

Mary Ann Hovis
Chair of Mathematics
Rhodes State College
4240 Campus Drive
Lima, OH 45804
(419) 995-8422
hovis.ma@rhodesstate.edu

Robert Kimball
Department Chair Math/Physics
Wake Technical Community College
9101 Fayetteville Road
Raleigh NC 27603-5696
(919) 662-3602
rkimball@waketech.edu

Rodney Null
Associate Professor of Mathematics
Rhodes State College
4240 Campus Drive
Lima OH 45804
(419) 995-8239
null.r@rhodesstate.edu

John C. Peterson
Professor of Mathematics (retired)
Chattanooga State Technical Community College
908 Westmoreland Blvd.
Knoxville TN 37919-7213
(865) 531-7455
john.peterson20@comcast.net

Jesse Williford
Instructor of Mathematics and Physics
Wake Technical Community College
9101 Fayetteville Road
Raleigh NC 27603-5696
919) 662-3637
ljwillif@waketech.edu

Program Officer

Elizabeth Teles
Lead Program Director for Advanced Technological Education
National Science Foundation
4201 Wilson Blvd., Room 835
Arlington, VA 22230
(703) 292-4643
ejteles@nsf.gov

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Glossary

10-fold dilution: 1/10th the concentration of the previous dilution. Each subsequent dilution is made from a previous dilution, so a 10-fold serial dilution is a series of 10-fold dilutions.

Absorbance, A: A measure of light absorption by a substance. In biotechnology, absorbance is often measured using an automatic plate reader.

Assay: An experimental procedure that measures a specific end point. An enzyme assay is a procedure that follows the activity of the enzyme, that is, the conversion of substrate to product by the enzyme. The assay tube will typically contain the substrate, a source of the enzyme, and a buffer or solution that provides a controlled environment in which the enzyme reaction occurs.

Beer-Lambert equation: The equation states that $A_\lambda = \lambda bC$, where A_λ is the sample's Absorbance value at a specific wavelength (or frequency), λ is the molar absorptivity coefficient of the material at that wavelength, expressed as L/moles-cm, b is the path length through the sample (usually 1 cm) and C is the concentration of the substance in moles/L.

Creatine kinase: An enzyme that is fairly ubiquitous in the body tissues. However, only brain and muscle tissue appear to release this enzyme into the bloodstream following injury. Therefore, creatine kinase measurement has been used to assess myocardial damage that may result from certain types of disease states.

Deoxyribonucleic Acid (DNA): Discovered by Frederick Miescher in 1869, it is the chemical basis for genes. The chemical building blocks (molecules) of which genes (i.e., paired nucleotide units that code for a protein to be produced by a cell's machinery, such as its ribosomes) are constructed. Every inherited characteristic has its origin somewhere in the code of the organism's complement of DNA. The code is made up of subunits, called nucleic acids. The sequence of the four nucleic acids is interpreted by certain molecular machines (systems) to produce the required proteins of which the organism is composed.

ELISA: The purpose of an ELISA (Enzyme-Linked ImmunoSorbant Assay) is to determine if a particular protein is present in a sample and if so, how much. There are two main variations on this method: you can determine how much antibody is in a sample, or you can determine how much protein is bound by an antibody. The distinction is whether you are trying to quantify an antibody or some other protein.

Endotoxin: A lipopolysaccharide (fat/sugar complex; poison, also known as LPS) which forms an integral part of the cell wall of gram negative bacteria. It is only released when the cell is ruptured. It can cause, among other things, septic shock and tissue damage. Pharmaceutical preparations are routinely tested for the presence of endotoxins. This is one reason why pharmaceuticals must be prepared in a sterile environment.

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Gram molecular weight of a substance: The weight in grams of one mole of the substance, and a 1 **molar** (1 M) solution by definition contains 1 mole of the solute in 1 liter of solution.

Hemacytometer: (also spelled hemocytometer) A cell counting chamber used with a microscope. It is an etched glass chamber with raised sides that will hold a quartz coverslip exactly 0.1 mm above the chamber floor. The counting chamber is etched in a total surface area of $9\,\mathrm{mm}^2$.

Limulus Amebocyte Lysate Test (LAL): A LAL test involves testing the liquid sample or the sample extract with Limulus Amebocyte Lysate (LAL). LAL is an aqueous extract of the blood cells of horseshoe crabs which forms a clot or change in color, depending on the technique, in the presence of bacterial endotoxin. The test sample is compared to a standard series of Control Standard Endotoxin (CSE) dilutions. The endpoint or reaction times of these dilutions are used to calculate the amount of endotoxin present in the sample. All tests are performed in at least duplicate. A positive control of the sample and negative control using non-pyrogenic water are also performed. An initial validation on 3 lots must be performed to validate this method of testing for a given product.

Order of magnitude: An exponential change of plus-or-minus 1 in the value of a quantity or unit. The term is generally used in conjunction with power-of-10 scientific notation. In base 10 an increase of one order of magnitude is the same as multiplying a quantity by 10. An increase of two orders of magnitude is the equivalent of multiplying by 100, or 10^2. In general, an **increase** of n orders of magnitude is the equivalent of multiplying a quantity by 10^n. As values get smaller, a decrease of one order of magnitude is the same as multiplying a quantity by 0.1. A decrease of two orders of magnitude is the equivalent of multiplying by 0.01, or 10^{-2}. In general, a **decrease** of n orders of magnitude is the equivalent of multiplying a quantity by 10^{-n}.

Phthalates: Chemical compounds that make plastics flexible and are used in many products including food wrap. The dozen or so types in general use today have some traits in common—they are clear liquids resembling common vegetable oil, have little or no smell, and do not readily evaporate. They all break down rapidly in the environment and in living organisms.

Spectrophotometer: A machine used to measure how much light of a given wavelength is absorbed by a liquid sample, or to measure how intense (bright) the spectral lines and bands produced by the sample are, relative to each other.

Stock Solution: A concentrated solution which is diluted to a strength that can be used.

Transmittance, *T*: The intensity of the light transmitted through a sample cell in a spectrophotometer. Transmittance may be expressed as a percent.

Tris: Tris is a base, and addition of Tris to a solution results in a pH > 10. The pH can be lowered to the desired value using a solution of hydrochloric acid (HCl). Beta mercaptoethanol has a density of 1.114 g/1 mL, which can be obtained from the Material Safety Data Sheet that is provided by the manufacturer, or from the *Merck Index*.

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