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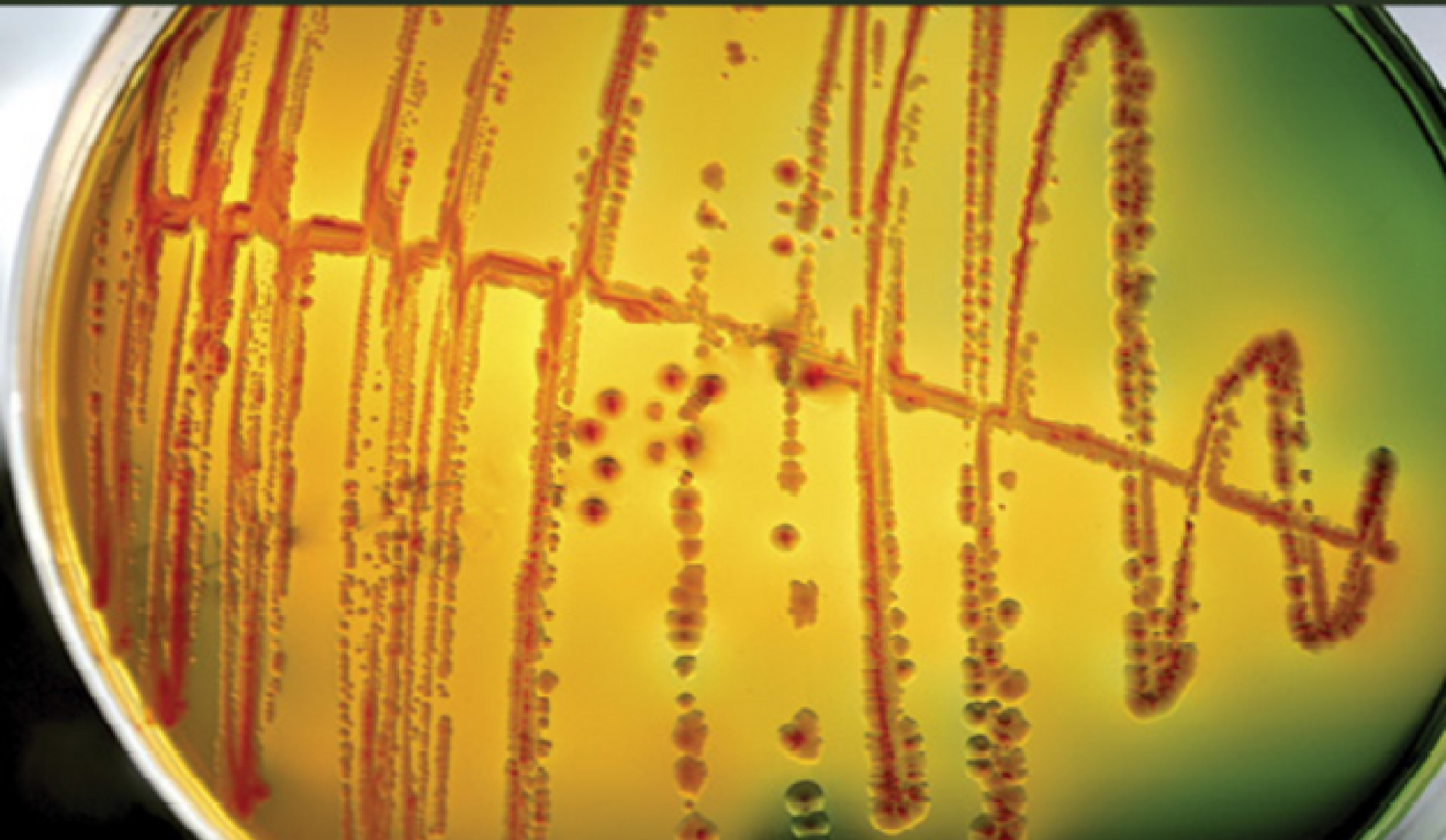
The Fundamentals of SCIENTIFIC RESEARCH

An Introductory Laboratory Manual



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Preface

In 2011, major stakeholders in the life sciences published a document entitled “Vision and Change in Undergraduate Education: A Call to Action” (AAAS, 2011). This document proposes a new way to teach undergraduate life sciences majors. The report suggests that undergraduate educators directly engage their students in the process of science by providing them with authentic experiences that mimic what we do as professional scientists. They postulated that biological content and the skills commonly associated with successful scientists can be introduced to students through meaningful evaluations of real biological phenomena.

The goal of the experiments in this laboratory manual is to introduce undergraduate students to the process of science through a guided introductory laboratory experience. Highlights of this manual include a semester-long experience working with one organism using experiments directed toward a single unifying goal, pre- and postlaboratory assignments and laboratory reports aimed to enhance the students’ analytical, critical thinking and scientific writing skills, and experience using common laboratory equipment and techniques similar to what are used in the professional setting.

Specifically, the laboratory curriculum centers on studying *Serratia marcescens*. *S. marcescens* is a Gram-negative bacterium that is unique in that it produces a red pigment, prodigiosin, at high cell density. It has been demonstrated that prodigiosin has several interesting properties; it is antimicrobial, it has been shown to induce apoptosis in cancer cells, and it has been demonstrated that it has potential to act as an immunosuppressant (reviewed in Khanafari *et al.*, 2006). There are currently many research laboratories that are attempting to enhance the production of prodigiosin because of these unique properties. The overarching goal of the laboratory course described in this manual is to have the students learn about the organism so that they can generate and initially characterize mutants of the organism that overproduce the pigment.

The laboratory manual breaks down the laboratory course into three separate modules. For the first module, the students familiarize themselves with common laboratory equipment and techniques. For the second module, the students begin to work with and appreciate *S. marcescens* by performing growth curves and Lowry protein assays, quantifying prodigiosin and ATP production, and performing complementation studies to understand the biochemical pathway responsible for prodigiosin production. They learn how to employ Microsoft Excel to prepare and present their data in graphic format and how to use specific calculations to convert their data into meaningful numbers that can be compared across experiments. The third module requires that the students employ UV mutagenesis to generate hyperpigmented mutants of *S. marcescens* for further characterization. They use experimental data and protocols they learned during the first and second modules of the course to help them develop their own hypotheses and experimental protocols and to help them analyze their data.

For each laboratory session, students are required to answer pre-laboratory questions that are designed to probe their understanding of the required prelaboratory reading materials (which includes the experimental background and protocol for that session and, in some cases, relevant primary scientific literature related to the experiments they will be performing in the laboratory). The questions also guide the students through the development of hypotheses and predictions. Following each laboratory, the students are required to answer a series of postlaboratory questions to guide them through the presentation of their data, analysis of their data, and placing of their data into the context of the primary literature. They are also asked to review their initial hypotheses and predictions to determine if their conclusions support their initial assertions. If their conclusions do not support their initial assertions, the students are asked to provide possible explanations as to why they think their conclusions did not agree with their hypotheses. The pre-laboratory and postlaboratory questions were designed to assist the students with the preparation of two formal laboratory reports after the second and third modules. The format for the reports is similar to that of primary scientific literature.

This laboratory manual seeks to introduce introductory undergraduate life sciences majors to an environment that fosters the development of scientific curiosity, creativity, and the critical thinking and communication skills required for success in the scientific disciplines. The laboratory techniques and skills that they will master through the exercises presented herein will provide the students with a strong foundation in the practice and process of science. All of these, in turn, should enable the students to persist and succeed as life sciences majors.

Acknowledgments

This work would not be possible if it was not for the students, faculty, and staff in the Department of Biology and Health Sciences–NYC. The feedback and support provided during the development of this work have been immeasurable and greatly appreciated.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com\go\kelly\fundamentals

The website includes:

- Instructor's Companion to the Lab Manual

Introduction

As life scientists, we are uniquely positioned to use our inquisitive natures to ask questions about the world around us. We have developed a systematic method to address the questions we pose. This method, the scientific method, is the major tenet behind what we do. The scientific method is as follows:

1. Make an observation.
2. Ask a question based upon your observation.
3. Develop a hypothesis.
4. Develop experiments to test your hypothesis.
5. Collect and analyze experimental data.
6. Confirm hypothesis or develop and test a new hypothesis.

You have probably memorized these steps at some point during your academic career, but have you ever *really* put them to the test to answer a biological question that has not yet been addressed? If not, you will gain practical experience with scientific method in this laboratory. If you have practiced the scientific method in the past, this laboratory will help you hone your skills.

Throughout the semester, you are going to be studying a single organism, *Serratia marcescens*. *S. marcescens* is a bacterium. Bacteria are considered prokaryotic organisms and have features commonly associated with prokaryotic cells. *S. marcescens* is classified as a Gram-negative bacterium because of the structure of its cell wall. Gram-negative bacteria have thin cell walls with phospholipid bilayers on both sides of the cell wall.

S. marcescens is unique among bacteria because it produces a red pigment called prodigiosin. Although the exact biological role of the pigment with respect to the organism is unknown, several laboratories throughout the world are studying the pigment and its activities. We are going to perform our own experiments in this laboratory course to understand the pigment and its biological significance. Ultimately, *your goal for this laboratory course will be to attempt to maximize the production of prodigiosin by hyperpigmented mutants that you will be creating.*

S. marcescens is ubiquitous in the environment. It preferentially grows in damp environments and is commonly seen in homes on bathroom tile grout and shower corners and at the toilet water line. When found, it appears as a pink, slimy film that is difficult to remove. In the bathroom, it primarily grows off soap and shampoo residues. Bleach-based disinfectants are typically recommended to completely remove the organism.

S. marcescens has left its mark on history several times. The Miracle of Bolsena, which occurred in the 13th century, is now attributed to *S. marcescens* contamination of the Roman Catholic host during the celebration of the Eucharist. In the Roman Catholic Church, when a priest blesses the bread and wine during the celebration of the Eucharist part of the Catholic Mass, they are believed to be transubstantiated into the body and blood of Jesus Christ. In 1263, a German priest who was celebrating Mass in Bolsena, Italy, noticed that “blood” was dripping from the host onto his linen robe and the altar linens. He attempted to clean his fingers and the altar and ended up smearing the linens further. He then took the bloodstained linens and the host to Pope Urban IV for investigation. The Pope perceived the event as a miracle that supported transubstantiation and, in honor of the miracle, created the Festival of Corpus Christi. Corpus Christi is celebrated in the Roman Catholic Church to this day.

Several microbiologists have simulated the Miracle of Bolsena on Manischewitz Passover matzos and unconsecrated wafers from both the Episcopalian and Roman Catholic churches. All samples from all laboratories involved in the study had *S. marcescens* growth on them that resembled blood (reviewed in Bennett, 1994 and Cullen, 1994).

Until the 1950s, it was believed that *S. marcescens* did not cause disease in humans—it was considered to be non-pathogenic. The US military is responsible for demonstrating that *S. marcescens* does indeed cause an atypical pneumonia in immune-compromised individuals (such as those individuals with active AIDS). The United States had an active biological weapons program from 1943 to 1969. In 1969, President Nixon declared that "… biological weapons are tactically inadequate…." He unconditionally and unilaterally renounced the testing and development of biological weapons and toxin agents—effectively shutting down the entire US program.

One reason Nixon shut down the program was from data obtained from several experiments that the US military performed on unsuspecting US populations. One such experiment, Operation Sea-Spray, was performed on September 26–27, 1950. For this experiment, the US Navy grew up large quantities of *S. marcescens*, aerosolized them, put them in balloons, and released them from barges located in the San Francisco Bay. The experiment was designed to test the effectiveness of bacterial aerosolization, the usage of balloons as biological weapons delivery systems, and the impact of wind current on dispersal distance. The US Navy selected *S. marcescens* for this test because the red pigment (prodigiosin) produced by the organism is easy to track. From this experiment, the US military concluded in a classified report that “It was noted that a successful BW [biological warfare] attack on this area can be launched from the sea, and that effective dosages can be produced over relatively large areas….”

The US military considered the experiment a success until beginning on September 29, 1950, there was a dramatic increase in the number of patients admitted to Stanford University Hospital with atypical pneumonias. Because the number of patients presenting with the atypical pneumonias continued to escalate in the following days, the US military performed a follow-up study. They demonstrated that the atypical pneumonias were all caused by the *S. marcescens* that they had sprayed over the San Francisco population. One patient, Edward J. Nevin, died from the *S. marcescens* atypical pneumonia. His family attempted to sue the US government, but the court decided that the government could not be sued because the spraying of *S. marcescens* was part of national defense planning (Cole, 2001).

S. marcescens is a very easy organism to work with. It is extremely versatile and its ability to produce prodigiosin makes it easy to detect. The laboratory course is broken down into three modules. For the first module, you will gain appreciation for and experience with common laboratory equipment and techniques that you will need for the rest of the course. For the second module, you will perform experiments to understand how *S. marcescens* grows, what it needs to grow optimally, and how and when it produces prodigiosin. You will also learn the details about prodigiosin biosynthesis and some of the biologically relevant characteristics of the molecule. For the third module, you will generate prodigiosin hyperpigmented mutants using UV light, and you will design your own experiments to initially characterize those mutants. *Your goal for the semester is to use what you learned in modules 1 and 2 to generate a hyperpigmented strain of* *<u>S. marcescens</u>* *for which you have determined the optimal conditions required for enhanced prodigiosin production.*

Using *S. marcescens* as a model organism, you will begin to build the skills to be successful undergraduate scientists. You must commit to think like a scientist by following the scientific method. Additionally, you will learn how to analyze your data and report your findings through the many writing assignments required for the course. Taken together, the work you will perform for this laboratory will provide you with a foundation for your future success as a life sciences major.

MODULE 1
Working with and Learning About Common Laboratory Techniques and Equipment

NAME ____________________ *DATE* ___________

Exercise 1A: Using Common Laboratory Tools to Evaluate Measurements Pre-laboratory Thinking Questions

Directions

Read over the introduction and protocols for this laboratory exercise and answer the following questions to ensure that you are prepared for the session:

1. What are the objectives for today’s laboratory (provide a numbered list)?
2. Why is it important to understand how to convert between metric units in the laboratory?
3. Why is it important to understand how to use serological pipettes and micropipettes (what will you be using them for in the lab—the more specific, the better)?

NAME ____________________ DATE ___________

Exercise 1B: Using Common Laboratory Tools to Evaluate Measurements

Measurements Introduction

Based upon modifications of worksheets developed by Susan Peckham Petro, DVM.

As scientists, many of the observations we make require that we take and compare measurements. For example, in the experiment you will perform today, you will take several measurements. You will measure different volumes of water using serological pipettes and micropipettes, you will measure the mass of the water using a balance, and you will use the density of water to help you calculate percent error. As we progress throughout the semester, you will continue to realize the importance of measurement taking. We primarily use the metric system to take our measurements. The metric system, developed in France in 1791, is based on units of 10. Fractions or multiples of the standard units of length, volume, and mass have been assigned specific names. The commonly used units of the metric system are highlighted in Table 1.1. Table 1.2 shows the prefixes used to designate fractions and multiples of these commonly used units and provides examples of how they are used.

Table 1.1 Commonly used units of the metric system.

Source: Susan Peckham Petro, DVM.

Measurement	Unit
Length	Meter (m)
Volume	Liter (l)
Mass	Gram (g)
Molar	Concentration (M)

Table 1.2 Prefixes used to designate fractions and multiples of these commonly used units.

Source: Susan Peckham Petro, DVM.

Fraction or multiple	Prefix	Symbol	Common usage of prefix
1×10^{6}—one million	Mega	M	.
1×10^{3}—one thousand	Kilo	K	.
1×10^{-1}—one tenth	Deci	D	Decade has 10 years
1×10^{-2}—one hundredth	Centi	c	Century has 100 years
1×10^{-3}—one thousandth	Milli	m	Millennium has 1000 years
1×10^{-6}—one millionth	Micro	µ	.
1×10^{-9}—one billionth	Nano	n	.

Often, upon taking a measurement, you will be required to convert that measurement to units that are different than the ones you used to initially take the measurement.

To Convert Smaller Units to Larger Units: Divide by the appropriate factor of 10 because there are fewer of the larger units.

Example 1: According to Table 1.2, a millimeter (milli = one thousandth) is 10 times smaller than a centimeter (centi = one hundredth). To change 1 millimeter (mm) to centimeters (cm), you must divide 1 by 10. So 1 mm is equivalent to 0.1 cm.

Example 2: According to Table 1.2, a nanogram (nano = billionth) is 1000 times smaller than a microgram (micro = millionth); therefore, to change 1 nanogram (ng) to micrograms (ug), you must divide 1 by 1000. So 1 ng is equivalent to 0.001 ug.

To Convert Larger Units to Smaller Units: Multiply by the appropriate factor of 10 because there will be more of the smaller units.

Example 1: According to Table 1.2, a decimeter (deci = one tenth) is 100,000 times bigger than a micrometer (micro = one millionth); therefore, to convert 1 decimeter (dm) to micrometers (um), you must multiply 1 by 100,000. So 1 dm is equivalent to 100,000 um.

Example 2: According to Table 1.2, a meter is 1000 times larger than a millimeter (milli = thousandth); therefore, to convert 54 meters (m) to millimeters (mm), you must multiply 54 by 1000. So 54 m is equivalent to 54,000 mm.

The Shortcut for Metric Conversions: The shortcut is simple—move the decimal points! When you realize that the units are 1000-fold apart, you can move the decimal over three places (for the three 0's) and change the units. Make sure that you are moving the decimal point the correct way when you do this:

If you convert from a larger unit to a smaller unit, move the decimal to the right. For example, if you want to convert 2.001 milliliters (ml) to microliters (ul), you must first recognize that there is a 1000-fold (1×10^3) difference between the two units and then move the decimal place three positions to the right (one position for each 0 in 1000) as indicated in the equation below:

Equation 1.1

Metric conversion from larger units to smaller units.

$$2.001\ \text{ml} = 2001\ \text{ul}$$

If you convert from a smaller unit (e.g., ng) to a larger unit (e.g., gram), move the decimal to the left. For example, if you want to convert 51,000 nanograms (ng) to grams (g), you must first recognize that there is a one billion-fold (1×10^9) difference between the two units and then move the decimal place nine positions to the left (one position for each 0 in one billion; you will have to add 0's to the left side of the number) as indicated in the equation below:

Equation 1.2

Metric conversion from smaller units to larger units.

$$000051{,}000.\ \text{ng} = 0.000051\ \text{g}$$

S*cientific Notation*: As you can see from some of the examples above, some of the numbers have many digits.

Scientific notation is a method used by scientists to simplify those numbers. Let’s convert the numbers provided in the metric conversion shortcut examples above to scientific notation.

To convert a whole number to scientific notation, place a decimal point to the right of the first digit in the number. To determine the exponent, count the number of digits to the left of the decimal point you just added. For example:

Equation 1.3

Conversion of a whole number into scientific notation.

$$2001\,\text{ul} = 2.001 \times 10^{3}\ \text{ul}$$

To convert a decimal number to scientific notation, place a new decimal point to the right of the first whole digit in the decimal. To determine the exponent, count the number of digits between the old and new decimal point. The exponent should be a negative number. For example:

Equation 1.4

Conversion of a decimal into scientific notation.

$$0.000051\,\text{g} = 5.1 \times 10^{-5}\ \text{g}$$

Significant Figures: Significant figures are the number of digits required to express the result of a measurement so that only the last digit in the number is in question. This means that when you are recording measurements, you should include all of the digits you are sure of *plus* a rounded estimate of the next smaller digit to the nearest tenth. In practice for the exercises in this laboratory manual, use, at most, three significant figures when reporting measurements. Below, please find some rules for determining the number of significant figures in a measurement:

1. The number of significant figures does not change when the decimal point is moved.

   Example: 625.2 m written as 0.6252 km—both have four significant figures

2. Zeros between two significant digits are always significant.

   Examples: 5.00004 has six significant figures, 94203 has five significant figures, and 650.007 has six significant figures

3. Trailing zeros to the right of the decimal point are significant in every measurement.

   Examples: 5.00 has three significant figures, 27.0 has three significant figures, and 30000.0 has six significant figures (You need to consider rules 2 and 3 for this last example as well, do you know why?).

4. Leading zeros are not significant in any measurement.

   Examples: 0.00007 has one significant figure, 0.708 has three significant figures, and 0.07808 has four significant figures (You need to consider rules 2 and 3 for this last example as well, do you know why?).

5. Trailing zeros appearing to the left of the decimal point may not be significant.

   Examples: 500 has at least one significant figure, 54640 has at least four significant figures, and 87,090,000,000 has at least four significant figures.

6. Any zeros that disappear when you convert a measurement to scientific notation are not significant.

Examples: 4000 converted to scientific notation (4×10^3) has at least one significant figure, 0.00064 converted to scientific notation (6.4×10^{-4}) has two significant figures, 260.00400 converted to scientific notation (2.6000400×10^2) has eight significant figures, and 0.008090 converted to scientific notation (8.090×10^{-3}) has four significant figures.

Let’s Put This to Practice!

Work independently to perform the following metric conversions. Your answers, where appropriate, should include three significant figures and be written out in scientific notation. After you complete the following problems, work with your laboratory group to come to a consensus for each answer. Be sure everyone in your group understands how each answer was determined. Finally, discuss your results with the class.

Convert 100 um to mm.

Convert 251 mg to g.

How many ug are in 354 kg?

How many seconds are in 2.567 ns?

Which is smaller—ul or ml?

Which concentration is higher—M or mM?

Which is larger—nm or mm?

Write 25 ug in g.

Convert 0.075 ml to ul.

Convert 659 nm to um.

Working with Serological Pipettes and Micropipettes

A vast majority of the exercises that you will work on in this laboratory require that you are proficient in using different tools to measure and dispense specific volumes of liquids. These tools include disposable serological pipettes and micropipettes.

There are four types of serological pipettes that you will be using in the laboratory based upon the total volume that they can draw up: 2, 5, 10, and 25 ml. Each of the four serological pipettes is calibrated so that you might be able to ensure that you draw up the appropriate volume of fluid as per the protocol you are working with. In order to draw up fluid, a pipette pump is attached to the top of the appropriate pipette to provide suction. Your laboratory instructor will demonstrate how to attach the pipette pumps and use the serological pipettes. Figure 1.1 provides examples of serological pipettes with attached pipette pumps.