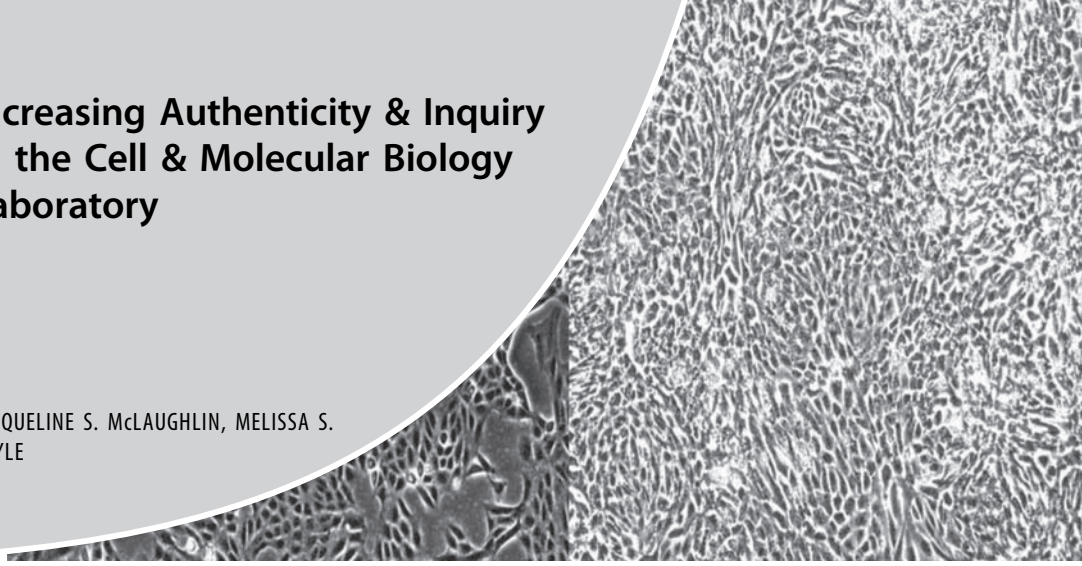
INQUIRY & INVESTIGATION

Increasing Authenticity & Inquiry in the Cell & Molecular Biology Laboratory

JACQUELINE S. McLAUGHLIN, MELISSA S. COYLE



Abstract

This article features a four-step pedagogical framework that can be used to transform the undergraduate biology laboratory into an authentic research experience. The framework utilizes a four-step scaffolding structure that not only guides students through the process of science and helps them gain mastery of relevant scientific practices, but also simplifies and streamlines the instructor's process of designing and implementing an authentic research experience in a biology lab course. We used this pedagogical framework to design an authentic research experience in which students investigated various factors affecting the growth and viability of a mammalian cell line, Vero cells isolated from kidney epithelial cells extracted from an African green monkey. Although this particular lab was designed for a cell and molecular biology course for university sophomores, the flexibility built into the pedagogical framework allows it to be used to design research experiences that can be implemented within a wide variety of lab courses at varying levels, effectively increasing the amount of authentic research experiences in biology lab courses nationwide.

Key Words: *Inquiry-based scientific research; authentic research; cell and molecular biology laboratory; cell culture; undergraduate research.*

○ Introduction

We are continuing to face new and increasingly complex environmental, social, and human health challenges on local, regional, and global scales in this 21st century. To effectively identify and address these current and impending issues, we, as educators, must prepare members of our society – scientists and nonscientists alike – with not only important content knowledge, but also the essential skills necessary to tackle important global issues. Those skills include the ability to find and critically evaluate information, to address and creatively solve problems, to communicate effectively, to collaborate within diverse groups, and to effectively use appropriate technologies to obtain, analyze, and transfer information. Authentic research experiences provide students with the opportunity to develop content knowledge and expertise in important scientific practices while also challenging them to relate these concepts and practices to larger, real-world issues or questions. Therefore, many prominent science-education agencies and organizations have recently called for more authentic research experiences in all science courses (Boyer Commission on Educating Undergraduates in the Research University, 1998; Project Kaleidoscope, 2002; National Research Council, 2003, 2005; National Academy of Sciences, 2010; AAAS, 2011, 2015).

We are continuing to face new and increasingly complex environmental, social, and human health challenges on local, regional, and global scales in this 21st century.

Although incorporating authentic research experiences in introductory biology laboratory classes is a great strategy to properly train students to tackle current and future challenges, problems exist for faculty implementation. Foremost are these: (1) the essential elements of an authentic research experience are unclear within the literature and between faculty, and (2) faculty generally lack the time necessary to develop and test new authentic research experiences for their students (Spell et al., 2014). Indeed, a national survey conducted by Spell et al. (2014) revealed that undergraduate students in 534 introductory biology courses spent, on average, only one-third of their time on authentic research activities, with 23% of courses having no research and 56% spending less than a quarter of total class time engaging in research.

One way to decrease the time spent in developing and implementing an authentic research experience that can be embedded within an introductory biology lab course is to follow a simple and flexible stepwise model. Although others have incorporated authentic experiences into introductory science lab courses (Grant & Vatnick, 1998; Gehring & Eastman, 2008;

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Weaver et al., 2008), what is lacking is a framework that includes the essential elements of authentic research and guides the instructor through the design and implementation process. In this article, we showcase a four-step pedagogical framework that does just that: it can be utilized by other faculty to more easily design and implement an authentic research-based lab experience in their unique institution and course.

Importantly, and for clarity, the essential elements of authentic research embraced by this framework include the following inquiry-based components: reading scientific literature, student-generated questions, hypothesis formation, experimental design, data collection and analysis, working toward significant findings, and oral and written presentation of results (Seago, 1992; Lopatto, 2003; Wiley & Stover, 2014). Additionally, from a skills perspective, this framework seeks to engage students in higher-order inquiry questioning (i.e., a fully implemented *open inquiry* wherein students ask questions whose answers are unknown; please review the scale of openness to inquiry-based learning initially devised by Schwab [1960] and later formalized by Herron [1971] – both of which served as a basis for defining the types of inquiry recognized today by the National Research Council [2000]). There is no information in any textbook, lab manual, or journal article about their expected results, because these are being discovered as part of the learning experience. This creates an environment in which students are participants in the development of new knowledge, and where the instructors are facilitating this process as research mentors (for an excellent explanation of how authentic research-based labs fill a niche in the transition from inquiry-based learning to professional science practice, see Weaver et al., 2008).

The flexibility built into this pedagogical framework makes it easily adaptable for a wide variety of lab courses at varying levels, as exemplified by the next article in this issue of *ABT*, "Student Scientists: Transforming the Undergraduate Biology Laboratory into a Research Experience" by Goedhart and McLaughlin.

○ Laboratory Pedagogical Framework

Figure 1 is a diagram of the four-step pedagogical framework used to simplify and streamline development of an authentic research experience that can be integrated into an undergraduate biology lab course. During step 1, students are taught essential and relevant experimental techniques via pre-lab videos and hands-on training (using the worksheets shown in Appendices 1–3). This step introduces students to critical elements of the scientific process and allows them to gain familiarity and practice with cutting-edge and pertinent scientific equipment and methods. Students are also introduced to the overall study system and its larger societal significance. This step can involve a small-scale and teacher-led project. During step 2, the instructor asks a large-scale guided question related to the study system. Students then work in small groups to research, select, and read primary literature related to the guided question in order to devise a more specific, self-directed research question (whose answer is unknown), formulate a hypothesis, and design an accompanying experiment to test the hypothesis. In step 3, students conduct their autonomous experiment while utilizing learned techniques and collecting data. During this step, an "open laboratory approach" is utilized – extra lab time is available and scheduled for student independent research on a daily basis, in addition to scheduled lab time (circumventing the "not enough lab time" hurdle for both faculty and students). Finally, in step 4, students interpret their data and present the results of their study in a professional scientific manner (i.e., poster, presentation, and/or scientific paper). Throughout this framework, instructors act as research chaperones, guiding student scientists through each step, providing constant feedback and environments that allow time for student self-reflection, mistakes, and dialogue over assignments before their final submission for grading (i.e., protocol, notebook, scientific paper, poster, etc.). Importantly, the four steps scaffold the scientific process, allowing students who are novices to the scientific process to progressively gain familiarity and comfort in inquiry-based skills. Moreover, this framework can

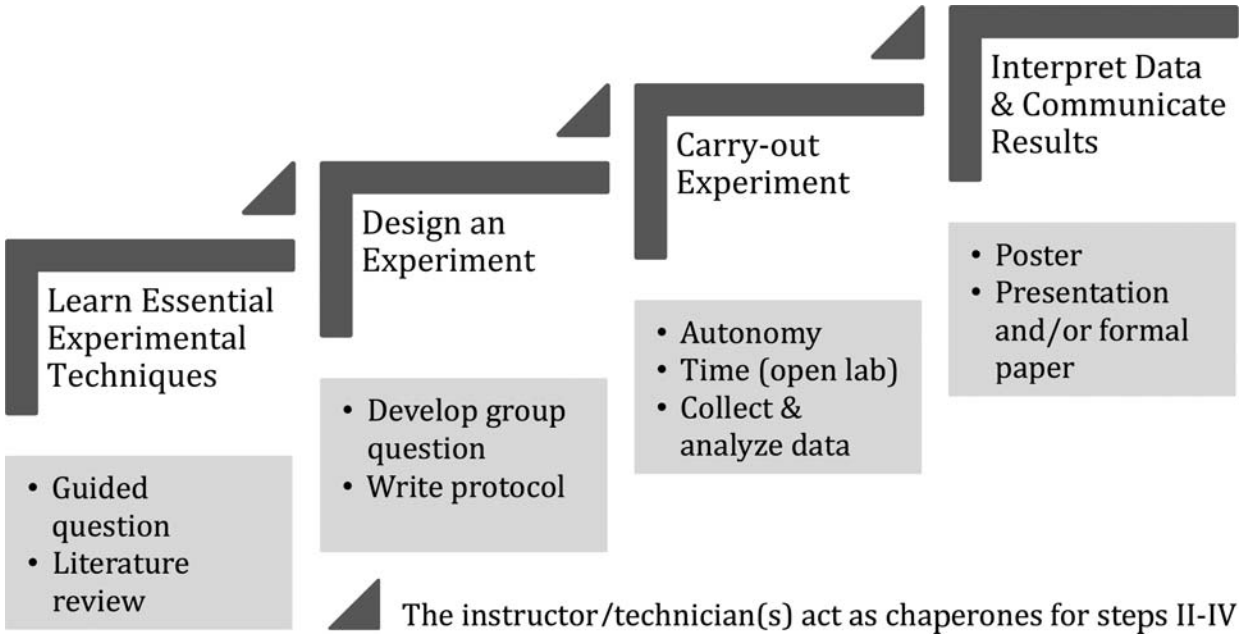

Figure 1. Pedagogical framework used to implement laboratory transformation.

last six weeks to an entire semester, depending on the needs of the students, instructors, or institution.

○ The Pedagogical Framework as Implemented in a Sophomore-Level Cell & Molecular Biology Lab Course at a Four-Year University

In an introductory cell and molecular biology lab at Pennsylvania State University–Lehigh Valley, a campus of ~1000 students, we used this pedagogical framework to design an authentic research experience in which sophomores investigated various student-selected factors affecting the growth of a mammalian cell line, Vero cells isolated from kidney epithelial cells extracted from an African green monkey (*Chlorocebus* sp.). In step 1, students were introduced to Vero cells and to the relevant scientific techniques associated with working with these types of cells, including sterile technique, cell passage, and cell counting. In step 2, students read articles from the primary literature and, working in groups, designed and executed an original experiment based on a unique research question related to the overarching, guiding question provided by the instructors: "What will happen to Vero cells that are grown in culture for eight days without a change of medium?" This question is relevant to present-day issues related to nutritional and/or physical stress of cells, extracellular matrix integrity, cellular junction functionality, and the importance of growth factors, tonicity, and pH for cell viability, both in culture (in vitro) and out of culture (in vivo). During step 3, students carried out their autonomous experiment by performing learned techniques and collecting data in a lab notebook using the "open laboratory approach." Finally, in step 4, students analyzed and presented the data in a scientific paper. Steps 1–4 took six weeks to complete under the direction of one faculty member and a laboratory research assistant.

○ Lab Protocol

Life & Death in the Cell Culture: An Undergraduate Lab in Cell Biology

1. **Objective**

   The key objective in this lab is to have you critically think about, and understand, "life at the cellular level." The cell is as fundamental to biology as the atom is to chemistry. Truly, everything that an organism does occurs, fundamentally, at the cellular level. Today's cell biologists are devising the newest medicines against cancer cells, cardiovascular disease, and hypertension; developing vaccines against viruses that invade normal, healthy cells; and growing stem cell lines in cell culture to differentiate neurons, blood cells, and muscle cells to help fight specific diseases.

   Video, "Stem Cells Breakthrough": http://www.pbs.org/wgbh/nova/body/stem-cells-breakthrough.html

   In this laboratory experience, you will investigate the factors that influence cellular life and growth. The cells under investigation will be placed in a fresh, nutrient-rich medium on day zero, and then allowed to grow in culture without a change of medium for eight days. Your task is to (1) hypothesize what will happen to these cells over the allotted time in cell culture, given limited nutrients, growth factors, buffering capacity, and space; (2) design and carry out an experiment, grounded in the primary literature, to test your hypothesis by utilizing cell culture techniques; and (3) interpret and then present your results in a formal research paper.

2. **Background Information**

   Animal or plant cells, after removal from specified tissues, will continue to grow if supplied with the appropriate nutrients and conditions. Cells that will grow for a limited number of time are known as *primary cells*. Cells that have the ability to proliferate indefinitely under appropriate conditions are known as *immortalized cells*. When this process is carried out in a lab, it is called *cell culture*. It occurs in vitro ("in glass or plastic") as opposed to in vivo ("in the multicellular organism"). The culture process allows single cells to act as independent units that "live off of" the nutrients supplied in a liquid or semi-liquid growth medium.

   Types of mammalian cultures. Cells can be removed from tissues in specific ways to be grown in cell culture. Often these cells remain under normal cell-cycle controls and maintain normal cellular senescence. These primary cells will have limited life spans, but they commonly maintain the original biology of the cell. Immortalized cell lines, on the other hand, retain the ability to divide indefinitely as a result of mutations (either natural or induced) that cause deregulation of the normal cell-cycle controls. These immortalized cell lines allow for cost-effective ways to study cells in culture and create reproducibility in scientific studies. A downside of these cell lines is that they have undergone various mutations to become immortal, which may alter the cells' life processes, and this must be taken into account when employing such cell lines. Yet most immortalized cell lines are well studied and characterized, which accounts for their wide use.

   When cells are grown in culture, they can grow as *adherent cells* (they "stick" to their culture vessel) or floating free in the culture medium as *suspension cells*. In either instance, the cells will continue to divide and grow until events such as a reduction of the necessary medium or space become a factor. At this point, cells will cease their division and growth and will need to be subcultured (aka "passed") to again initiate their growth and division. For cells grown in suspension, this is usually done by removing the cells from their culture flask, centrifuging them into a pellet, resuspending and simultaneously diluting them in fresh culture medium, and then placing them in a new flask and back into the incubator. Cells that grow adherently must first be treated with trypsin to detach the cells from their flask before performing the same steps as above.

   Video, "Sterile Technique": http://media.invitrogen.com.edgesuite.net/Cell-Culture/videos/SterileTechnique.html?CID=ccbvid2

   Video, "Passaging Cells": http://media.invitrogen.com.edgesuite.net/Cell-Culture/videos/4CellPassagingCells.html?CID=ccbvid3

Video, "Cell Counting": http://www.abnova.com/abvideo/Cell-Counting-Cell-Suspension.html

Cell culture conditions. To grow cells in cell culture is a complex process. Cells are typically maintained at an appropriate temperature and gas mixture (typically 37°C and 5% CO_2 for mammalian cells) in a carefully calibrated, and frequently checked, incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the medium. Recipes for media can vary in pH, glucose concentration, growth factors, buffers, electrolytes, amino acids, ion composition, and vitamins and minerals. Moreover, growth factors may be used to supplement media, and these are often derived from animal blood, such as fetal or calf serum. To cell culturists, an ideal cell culture environment is one that does more than just allow cells to increase in number by undergoing cell division (mitosis). Even better is an environment that allows for mitosis and cellular expression of in vivo physiological and cellular functions, such as hemoglobin expression in red blood cells or secretion of insulin by beta pancreatic cells.

Vero cells. Vero cells are epithelial cells that were derived from the kidney of a normal adult African green monkey in 1962 by Y. Yasumura and Y. Kawakita at Chiba University in Japan. Although these cells were derived from a normal kidney, they present with an abnormal chromosome number, or aneuploidy (ATCC, 2012). Because these adherent cells can be grown as a continuous cell line, they are commonly used in microbiology and cell biology research. Some of the more common applications include virology studies, viral vaccine production, toxicity studies, and propagation and study of intracellular bacteria and parasites (Ammerman et al., 2008).

3. Laboratory Assignment

1. Learn select experimental techniques, including the following: basic cell culture techniques; Vero cell culture conditions and passage; and cell counting (viable and nonviable cells) technique using a hemocytometer (Figure 2 and 3).
2. Read three peer-reviewed research articles (approved by your instructor) that pertain to the viability and growth dynamics of mammalian cells in tissue culture.
3. Devise a more concise research question and hypothesis to explain the changes (life vs. death) in freshly passed Vero cells at 10^5 cells/mL that remain in culture for an eight-day period without change of medium when all other culture conditions are kept constant. You may make alterations in the medium's components – such as glucose, glutamate, and specific growth factors – as long as you have the proper controls.
4. Envision a research strategy based on the above hypothesis that includes these components: Purpose (which includes background information, hypothesis under investigation, and scientific reasoning), Materials, Procedure, Data Interpretation, and References (using a standard "protocol" format). Importantly, on each day over an eight-day period, microscopic observations of Vero cell density and morphology, pH of medium, and counts of viable and dead cells must be noted.
5. Undertake the experiment according to the research design, collect data, and present research findings in the form of a scientific paper.

Website, "Scientific Writing":http://www2.lv.psu.edu/jxm57/irp/sciwrit.html

4. Techniques

I. Vero Cell Passage

Materials & Equipment:

- Vero cells, confluent monolayer, in tissue culture flask
- Growth medium, RPMI 1640 (1×), supplemented with 5% fetal bovine serum (FBS), 1% penicillin-streptomycin
- Trypsin-EDTA (1×) or cell scrapers
- Phosphate buffered saline (PBS) (1×), without calcium or magnesium
- 15 mL conical tubes, sterile
- 25 cm^2 tissue culture flasks, sterile (T25)
- Serological pipettes, sterile
- 70% ethanol solution (used for decontamination of hood and objects brought into the hood)
- Waterbath, setpoint 37°C
- CO_2 incubator, setpoint 37°C and 5% CO_2
- Inverted phase-contrast light microscope (used to check cell morphology)
- Biological safety cabinet (hood)

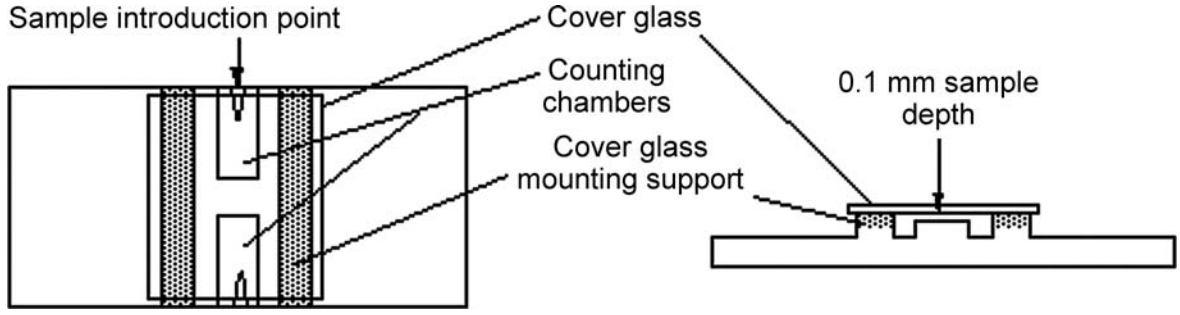

Figure 2. Diagram of hemocytometer (http://web.mnstate.edu/provost/CountingCellsHemocytometer.pdf).

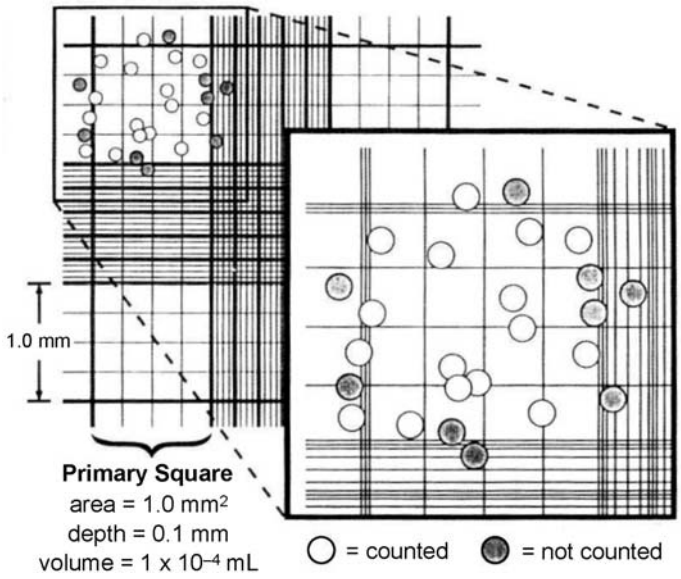

Figure 3. Counting cells and grids of a hemocytometer (http://web.mnstate.edu/provost/CountingCellsHemocytometer.pdf).

Procedure:

1. Remove growth medium from monolayer of Vero cells.
2. Wash flask twice with PBS (no Ca^{2+} or Mg^{2+}). Use 2 mL for each wash, making sure to wash the bottom of the flask. Decant wash into waste beaker.
3. Add 2 mL trypsin to the flask.
4. Rock the flask back and forth for 60 seconds. Put the flask in the 37°C incubator for 1–2 minutes.
5. Strike the flask sharply against the palm of the hand to dislodge the cells and then look at the flask under the microscope. If the cells remain attached, warm the flask in the palm of the hand for 30 seconds and then strike the flask again.
6. Add 4.5 mL fresh medium to the flask (the FBS in the growth medium inactivates the trypsin). Mix with pipette, then add the medium to a separate 15 mL centrifuge tube.
7. Centrifuge for 5 minutes at 500 rpm.
8. Remove and discard supernatant.
9. Resuspend cells in 5 mL RPMI with 5% FBS.
10. Prepare desired dilution of cells in RPMI with 5% FBS and add to 25 cm^2 (T25) cell culture flasks.
11. Incubate flasks in 37°C incubator with 5%CO_2.

Note: Monitor cells daily or every other day. Change media every few days. When cells reach a 70–90% confluent monolayer, passage cells again.

II. Vero Cell Counting Using a Hemocytometer
Materials & Equipment:

- Hemocytometer
- Weighted coverslip
- Trypan blue, 0.4% solution
- PBS (1×)
- 70% ethanol solution (to clean slide and coverslip)
- Lens wipes
- Microscope
- Pipette and tips
- Biological safety cabinet (hood)
- Centrifuge

Procedure:

1. Decant medium from Vero cell stock flask into 15 mL centrifuge tube.
2. Wash flask twice with PBS (no Ca^{2+} or Mg^{2+}). Use 2 mL for each wash, making sure to wash the bottom of the flask. Decant wash into waste beaker.
3. Add 2 mL trypsin to the flask.
4. Rock the flask back and forth for 60 seconds. Put the flask in the 37°C incubator for 1–2 minutes.
5. Strike the flask sharply against the palm of the hand to dislodge the cells and then look at the flask under the microscope. If the cells remain attached, warm the flask in the palm of the hand for 30 seconds and then strike the flask again.
6. Add 4.5 mL fresh medium to the flask (the FBS in the growth medium inactivates the trypsin). Mix with pipette, then add the medium to a separate 15 mL centrifuge tube.
7. Centrifuge for 5 minutes at 500 rpm. Pipette off the supernatant (discard) and resuspend the pellet in 1 mL growth medium. Transfer 1 mL cells to the microcentrifuge tube for cell count.
8. In a fresh dilution tube, mix 400 µL PBS 1× (or fresh cell growth medium), 50 µL trypan blue, and 50 µL cell suspension. This constitutes a tenfold dilution (dilution factor = 10).
9. Carefully load 10 µL to both sides of the hemocytometer (counting chamber). Take care not to flood either side of the slide.
10. Count and record the total number of live (clear) and dead (blue) cells in five squares. Repeat the process for the cells loaded on the other side of the hemocytometer.
11. Calculate the number of cells per milliliter, the number of live cells per flask, the number of dead cells per flask, and the percent viability.
12. Dispose of all cell culture flasks and pipettes in a biohazard bag.

Calculations:

$$\text{Cells/mL} = \frac{\text{Number of cells in squares}}{\text{Number of squares counted}} \times 10^4 \times \text{dilution factor}$$

$$\text{Percent viability} = \frac{\text{Number of unstained cells (live)}}{\text{Total number of cells}} \times 100$$

○ Conclusion

This example of an authentic, higher-end-inquiry, cell-culture research lab experience was designed to allow sophomore-level

biology major students to learn cutting-edge scientific practices while addressing current issues in the field of cell biology – nutritional and/or physical stress of cells as they relate to cell growth and viability. Guided through the four-step pedagogical framework, students work within groups to learn modern cellular biology techniques, ask their own questions, develop testable hypotheses based on information gathered from relevant scientific literature, devise and carry out a controlled experiment, and present the data in a professional scientific manner. What best exemplifies the authenticity of the research and the enhanced performance of the students who completed it is this: Many students who participated in the above assessment study went on to present their work at peer-reviewed conferences or to publish their research (e.g., Adams et al., 2015; Arguello & McLaughlin, 2016). These affirmative results are consistent with those of other studies that measured students' perceptions and attitudes (Howard & Miskowski, 2005; Weaver et al., 2008; Caruso et al., 2009; Harrison et al., 2011; Brownell et al., 2012), ability to design experiments and interpret data (Myers & Burgess, 2003), and information fluency (Gehring & Eastman, 2008) in lab courses that integrated authentic, research-based experiences into the curriculum (Goedhart & McLaughlin, 2015).

Additionally, the framework has sufficient flexibility to serve as a tool for transforming other laboratories and classrooms at various levels within a variety of institutions. These adaptations could include the study system, the guided-inquiry challenge question, the experimental techniques, the mode of data presentation, and the duration of the lab itself. (Please see Goedhart and McLaughlin's article, following this one, for an example of how this pedagogical framework can be adapted to accommodate a different student population; also see Goedhart and McLaughlin, 2015.) Widespread adoption and adaptation of this pedagogical framework has the potential to push us further toward the goal of more authentic research experiences in all science courses.

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Appendix 1

Determining Cell Confluency

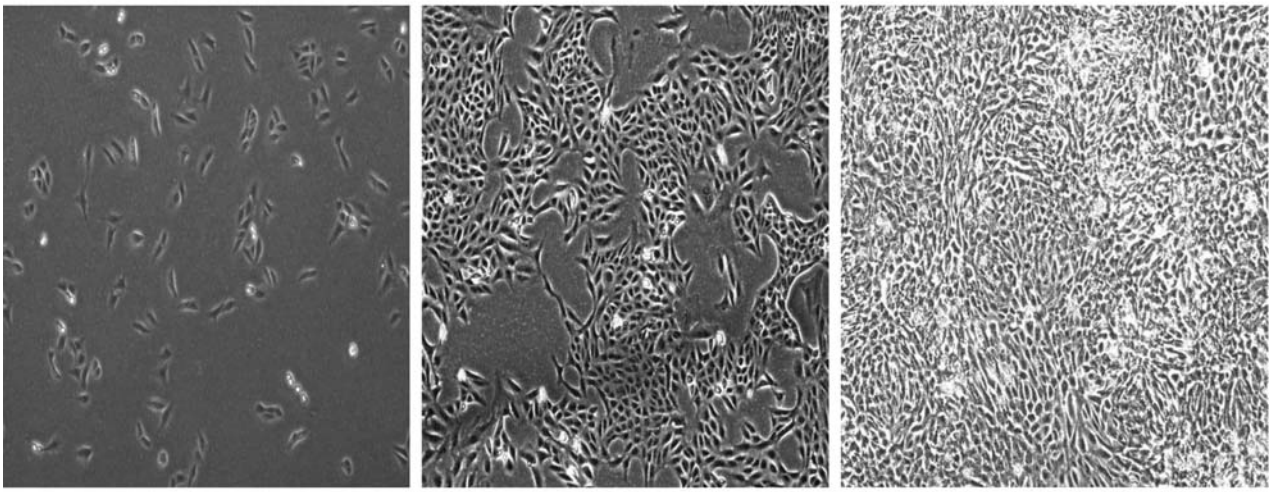
Penn State Lehigh Valley

Jacqueline S. McLaughlin and Melissa S. Coyle

Confluency of an adherent cell line refers to how much area of the cell culture flask or plate is covered with cells. It is used to determine when a cell line should be subcultured (or passed) for continued healthy cell growth. The optimal confluency for subculturing cells is approximately 70-80%:

- If subculture is performed too soon (i.e. low density), the cells will be in the lag phase of their growth curve and will be slow to proliferate
- If subculture is performed too late (i.e. high density), the cells will be in the stationary or declining phase of their growth curve. Once they've reached this point, they may undergo unfavorable transformation and may be difficult to remove from flask or dish

Label the approximate Vero cell confluency (inverted phase contrast microscope, 40x):



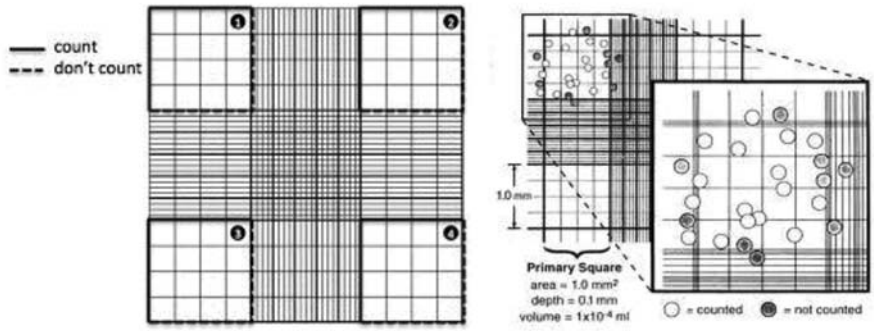
% Confluency = _____ % Confluency = _____ % Confluency = _____

Appendix 2

Counting Cells on a Hemocytometer

Penn State Lehigh Valley

Jacqueline S. McLaughlin and Melissa S. Coyle



http://web.mnstate.edu/provost/CountingCellsHemocytometer.pdf

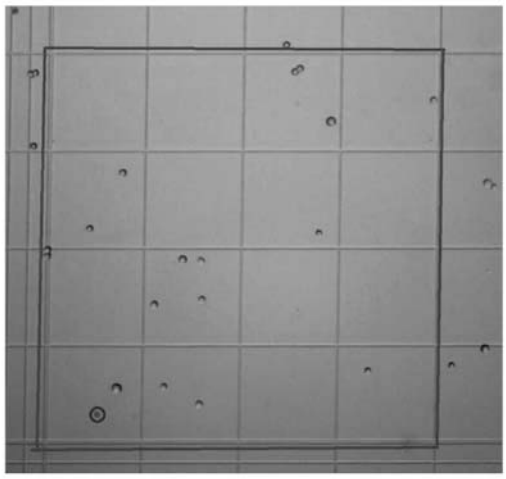
Note: Only count the cells touching the left and top borders on the 4 large squares (cells must be touching the middle line of the three to be counted).

Calculations:

$$[\text{cells/ml}] = \frac{(\text{Total \# cells counted})\,(10^4)\,(\text{dilution factor})}{(\text{Total \# squares counted})}$$

$$\%\ \text{viable cells} = \frac{(\text{\# Live cells counted}) \times (100)}{(\text{Total \# cells counted})}$$

Practice Problem: **Please note, this is only 1 square of the 4 large squares*



1. How many total cells are in the outlined red square? ____
2. How many are live? ___ How many are dead? ____
3. *What is the concentration of the cell culture if your cell count preparation was made by adding 50uL cells + 50uL trypan blue + 400 uL PBS?
4. What is the percent viability of the sample?

Appendix 3

Calculating the Concentration of Cells

Penn State Lehigh Valley

Jacqueline S. McLaughlin and Melissa S. Coyle

Calculation: used to calculate cells/mL using a hemocytometer

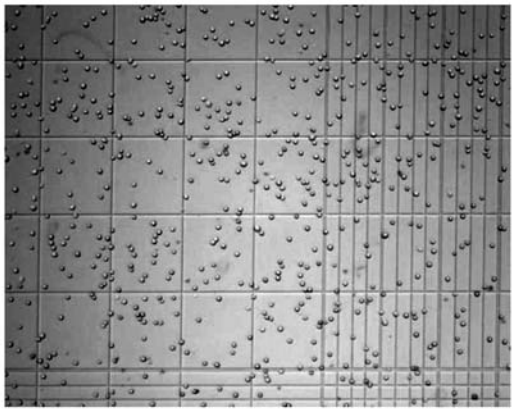
$$[\text{cells/mL}] \quad \frac{\text{(Total \# cells counted) } (10^4) \text{ (dilution factor)}}{\text{(Total \# squares counted)}}$$

Practice Calculation 1:

A student adds 50 µL cells to 50 µL trypan blue and 400 uL PBS (total volume is 500uL), then counts 120 cells in 4 squares of a hemocytometer. Hint: Since she has diluted her cells 10X (50uL cells into 500uL total volume); the dilution factor is 10. What is the cell concentration of her culture?

Practice Calculation 2:

A student sees the following number of cells in 1 large square of the hemocytometer and decides to prepare a more dilute sample by adding 50uL cells + 50uL trypan blue + 900uL PBS = 1000uL total volume.



With the new dilution, the student counts 103 cells in four squares of a hemocytometer. What is the cell concentration?