This article reprinted from:

Hoefnagels, M. H., and M. E. Walvoord. 2005. Conversion immersion: Working together to create investigative laboratories. Pages 111-120, *in* Tested Studies for Laboratory Teaching, Volume 26 (M.A. O'Donnell, Editor). Proceedings of the 26th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 452 pages.

Compilation copyright © 2005 by the Association for Biology Laboratory Education (ABLE) ISBN 1-890444-08-1

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the copyright owner. Use solely at one's own institution with no intent for profit is excluded from the preceding copyright restriction, unless otherwise noted on the copyright notice of the individual chapter in this volume. Proper credit to this publication must be included in your laboratory outline for each use; a sample citation is given above. Upon obtaining permission or with the "sole use at one's own institution" exclusion, ABLE strongly encourages individuals to use the exercises in this proceedings volume in their teaching program.

Although the laboratory exercises in this proceedings volume have been tested and due consideration has been given to safety, individuals performing these exercises must assume all responsibilities for risk. The Association for Biology Laboratory Education (ABLE) disclaims any liability with regards to safety in connection with the use of the exercises in this volume.

The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises.

Visit ABLE on the Web at: http://www.ableweb.org



Chapter 6

Conversion Immersion: Working Together to Create Investigative Laboratories

Mariëlle H. Hoefnagels and Mark E. Walvoord

Departments of Zoology and Botany-Microbiology University of Oklahoma Norman, OK 73019 hoefnagels@ou.edu oumadfrog@ou.edu

Mariëlle Hoefnagels is an assistant professor at the University of Oklahoma, where she teaches nonmajors biology, nonmajors microbiology, mycology, and a senior capstone course in zoology. She recently received the University's General Education teaching award. She is also co-author of the general biology textbook, *Life*. She received her Ph.D. in botany and plant pathology at Oregon State University.

Mark Walvoord is an instructor at the University of Oklahoma, where he also received his MS in Zoology. He teaches Introductory Zoology and has assisted with the administration of an additional 1,600 students per year in other sections of Introductory Zoology. Mark is interested in herpetology, ecology, and Madagascar.

© 2005, University of Oklahoma

Contents

Introduction 112
Workshop methods 112
Results of workshop sessions
1. Enzymes 113
2. Osmosis 113
3. Fermentation 114
4. Microscopy and Cells 114
5. DNA Isolation 115
6. Genetic Engineering: pGLO Transformation in Bacteria 115
7. Antimicrobial Agents 115
8. Effects of pH and Heavy Metals on Microbial Processes 116
9. Immunohistochemistry 116
10. Early Development of the Chick 117
11. Muscle Contraction 117
12. Invertebrate Diversity 117
13. Plant Diversity 118
14. Soils 118
Conclusions 119
Acknowledgements 120
Literature Cited 120

Introduction

Despite burgeoning interest in investigative laboratories, many student labs retain a "cookbook" format. That is, students follow a pre-determined "recipe," hope the results come out like their teacher told them it's supposed to, and leave the lab without having thought much about what they did or why they did it. In contrast, investigative labs are typically somewhat open-ended. They typically lead students to think of their own experimental variables, gather data, and arrive at a logical conclusion. Instead of rigid adherence to a defined procedure, investigative labs emphasize discovery, hypothesis formation and testing, and data evaluation.

The objective of the Conversion Immersion major workshop was to provide a forum for instructors to work together to generate ideas for modifying specific, traditional ("cookbook") labs to a more investigative format. The morning session had 23 participants; the afternoon session had 15. This paper summarizes some of the ideas that emerged from the two sessions and seeks to identify some common elements of converting labs.

Workshop methods

Workshop participants submitted "cookbook" labs to us a few weeks before the workshop. We summarized the labs and organized them into groups of related activities. On the day of the workshop, after an explanation of our purpose, we divided the participants into groups of four or five and assigned each group a set of labs. The participants spent about half of the workshop working in their groups,

113

brainstorming and summarizing their ideas for making the labs more investigative. Then each group reported its ideas to the rest of the workshop participants.

The summaries presented here represent a compilation of notes we took during the oral presentations and written notes that each group submitted. Many groups incorporated suggestions such as requiring students do research before the lab, submit brief experiment "proposals" so the instructor could gather required materials, and/or complete concept maps before or after the lab. Some recommended formal lab reports, posters, or oral presentations as a follow up to these student investigative labs. To save space, and because instructors could implement those measures in many different types of labs, we have omitted most of those suggestions from the summaries below. Exceptions appear where the exercise is integral to the lab procedure.

Results of workshop sessions

1. Enzymes

Original procedure: In this lab, students first test the effects of boiling, pH, and shaking on egg albumin. They then investigate the effects of various conditions (heat, HCl, NaOH, cofactor removal) on catecholase from potato extract. Next, they compare the action of catalase to an inorganic catalyst. They also investigate the substrate specificity of catecholase and the effect of cofactor (iron) removal on catecholase and catalase action.

Group suggestions: The group suggested that the scope of this lab be enlarged to include protein structure in general, and then divided into two questions: (1) how could you alter protein structure, and (2) how could you alter protein function?

For part 1, the group suggested using albumin, since its appearance changes when denatured. Students could add HCl, NaOH, or items from home that might affect protein structure. (The instructor should also provide items that do not affect protein structure.) For part 2, the group suggested using one or more enzymes suitable for easy, non-quantitative assays, including amylase, rennin, RNAse/RNA plates, Lactaid, or Beano. Although the students cannot watch the enzyme work, they can still measure its action. They could then investigate how the environment affects enzyme action. For example, they might see how the treatments that denatured albumin affect these enzymes. Students should also make the connection between enzyme action and the environment in which the enzyme naturally occurs (e.g., stomach vs. intestine). For example, rennin has a different pH optimum than other enzymes, and RNAse enzymes are heat stable.

This lab generated lively discussion, reflecting some ambiguity about the lab's objectives. One topic of concern was whether this type of lab really engages students in the underlying significance of enzyme function. Other workshop participants thought it might be better to engage students by challenging them with a realistic problem. For example, what is the best temperature to ferment beer (or yogurt or cheese) to get the best product as fast as possible?

2. Osmosis

Original procedure: Students learn the I_2KI and Benedict's tests for determining the movement of water and/or solute (starch or glucose) through dialysis tubing, observe the behavior of red blood cells placed in solutions of different osmolarity, and determine the effect of solute concentration on the rate of osmosis.

Group suggestions: The group assigned this lab did not have time to fully develop an idea, but the participants suggested having students predict the osmolarity of an unknown solution by observing its behavior relative to solutions of known osmolarity.

3. Fermentation

Original procedure: Students seal yeast cells in an airtight flask with a glucose solution. They test for CO_2 in the gas produced during fermentation, and test for ethanol in distillate collected from the incubation mixture. Students also estimate the concentration of glucose before and after the incubation period.

Group suggestions: The group suggested introducing the lab by presenting a case study, such as a bakery that can't get its dough to rise, or a brewery that needs to make beer quickly for a competition. After teaching students the techniques for measuring CO_2 volume (water displacement) or ethanol production (flames or chromic acid), instructors could challenge the class with the question: how could you make the fermentation go faster or produce more CO_2 or more ethanol? Students can then devise their own hypotheses. They could change the species, brand, or amount of yeast; the amount or type of sugar (e.g. sucrose, ribose, fructose, glucose); incubation pH; salt concentration; or fermentation temperature. The product of the lab could then be a written or oral report to the brewer or baker, recommending improved procedures for beer or bread production.

4. Microscopy and Cells

Original procedure: This group worked with two labs, entitled "Introduction to the Microscope" and "Cells: Diversity in Structure and Function." In the Microscopy lab, students learn about the parts and use of a microscope, then examine cheek cells, *Elodea* cells (with and without salt water), bacteria, and *Paramecium*. In the Cells lab, students observe and draw wet mounts and prepared slides of *Elodea* cells, fungi (*Rhizopus*), human epithelial cells, normal skin cells, basal cell carcinoma cells, and protists from pond water.

Group suggestions: This group suggested beginning with a pre-lab on microscope basics. This might include introducing the eyes, then the dissecting scope, then the compound scope, then the electron microscope. The group also suggested a game to introduce the microscope as a tool, in which students would sort objects of different sizes and measure specific objects.

The lab would then move to a case study in which students would use the microscope to solve a crime or medical problem. Useful case studies might include a hospital trying to determine the source of a deadly staph infection; an epidemic of disease among farm animals; a forensic examination (including insects) of a person's cause of death; or an environmental problem such as an algal bloom. Whatever the case study, the instructor would provide students with "evidence" in the form of debris, tissue samples, water samples, pathogen samples, photographs of a crime scene, electron micrographs, and the like. Dichotomous keys might also be useful. Students would observe the evidence and answer questions such as: "What are they?" "How big are they?" "Are they prokaryotes, eukaryotes, or viruses?" "If they are cells, what kind are they?" At the end of the lab, students might create a mock report to a boss or health commissioner, explaining their solution and reasoning.

The group also noted that this type of approach could easily apply to other types of labs as well. For example, with appropriate specimens, the instructor might ask "What plant part is this?" "What stage of

the life cycle is this in?" "Why is this microbe growing here now, but it didn't before?" "Why isn't this plant growing well?"

5. DNA Isolation

Original procedure: Students isolate nuclear DNA from wheat germ using a standard protocol. They also determine the effects of making various changes in the protocol (e.g. remove the protein, test the effect of incubation temperature, omit the detergent, etc.).

Group suggestions: The group suggested that the lab should challenge students to compete to achieve some objective. For example, the instructor might ask students to suppose a company had hired them to perfect their DNA isolation protocol. The question might become "How do you get as much purified DNA as possible from a sample?" The class could then look at a variety of DNA isolating protocols, identify the common steps, explain what each step is for, and consider which variables might affect purity and yield. Each group of students could then choose a DNA isolation protocol, select one or more variables to alter within the protocol, and design an experiment to test the effect of the variable(s) on DNA purity and yield. (The whole class would have to use the same procedure to measure purity and yield). A variation on this idea would be to have students use the same DNA isolation procedure, but each group would use a different type of organism. To complete the lab, students would make a flowchart showing the protocol and the changes they made, show the results, discuss the variables and their effects, and explain why a certain protocol would be best.

6. Genetic Engineering: pGLO Transformation in Bacteria

Original procedure: Students follow a defined series of steps to introduce the pGLO plasmid into *E. coli* cells and plate the cells (+ pGLO or - pGLO) on agar containing different antibiotics. After observing the results of different treatments, students calculate transformation efficiency.

Group suggestions: The group did not suggest specific procedures for making the lab investigative, but they did suggest three scenarios that would help give students a context for the steps in transformation. (1) You have been hired by a biotechnology company, and your first job is to improve the frequency of transformation of their *E. coli* cells. Which steps in the transformation protocol could you alter to improve the method? Background research is important here for two reasons. First, it will help students better understand the protocol. Second, this protocol already has low transformation efficiency, so blindly omitting certain steps will yield nothing. (2) The label has been washed off your two tubes of plasmid DNA. How would you find out which plasmids you have? (This lab would focus on the use of antibiotic markers to differentiate between plasmids, but the students would have to transform *E. coli* to figure out what each plasmid is.) (3) It may be possible to create two different plates of bacteria that both have been transformed with pGLO, but one glows more than the other. Students could then investigate factors that influence the glowing intensity in the cells (e.g. cell density, age of culture).

7. Antimicrobial Agents

Original procedure: Students explore the effects of antibiotics and antiseptics on the growth of gram-negative and gram-positive bacteria. They soak small circles of absorbent paper in the candidate antimicrobial agent and place them on a "lawn" of bacteria. In a subsequent lab, students determine whether the agent produced a clear "zone of inhibition" against the bacteria.

Group suggestions: The instructor could give students a list of bacteria to choose from and a list of possible inhibitors (e.g. medicinal plant extracts, salts, antiseptics, soaps, light, etc). Students could do background research, including an explanation of their hypothesis and a proposed experimental plan. Then students would set up their experiments, document the results (using digital photos, or zone of inhibition measurements), and write a scientific paper.

8. Effects of pH and Heavy Metals on Microbial Processes

Original procedure: This complex lab includes the following objectives (among others): to investigate the influence of acidification on ammonification and to determine the inhibitory effects of three heavy metal compounds on the growth of four bacterial species. Students expose bacterial cultures and intact soils to solutions of different pH, then measure ammonification in a later lab. They also soak paper disks in mercuric iodide, silver nitrate, or copper sulfate, place them on agar plates streaked with different bacteria, and measure zones of inhibition in a subsequent lab.

Group suggestions: The group thought this lab had too many objectives and focused too much on technique rather than the process of science. They thought it would be better to have students preresearch particular environmental parameters that they would like to investigate in the lab (e.g. pH, heavy metals, antimicrobial agents, temperature, agitation). They also suggested letting students develop their own methods instead of following the specific procedures the lab currently prescribes.

In follow-up comments, other workshop participants suggested having students isolate bacteria from habitats where microbes naturally encounter competition (e.g. lake water, rotten logs) and determine whether they inhibit each other. Another suggestion was to look at the effects of contaminants (e.g. environmental hormones from women on hormone replacement therapy) or to manipulate other environmental variables.

9. Immunohistochemistry

Original procedure: Students use anti-HNK1, an antibody that recognizes a protein present on the surfaces of migrating cells (primarily neural crest cells). The protocol includes directions for fixing correctly staged embryos and performing immunohistochemistry.

Group suggestions: The morning group pointed out that the lab as currently written does not ask a question that would motivate students to learn the tool of immunohistochemistry. Therefore, the group suggested that the lab become a multi-part series in which the students first learn why each step in the protocol is important. Students might research why investigators use certain reagents (rather than others), or they might present different elements of the protocol to the class. They could then do an experiment in which they use immunohistochemistry to compare what happens to the neural crest and/or muscle cells in a control organism and an altered one.

The afternoon group suggested a set of questions that students might investigate, noting that anti-HNK-1 reacts with proteins on the surfaces of migrating cells. The questions included: What other tissues or cells might react with the antibody? Which other cells migrate, and under what circumstances? Would this antibody react with migrating cells in other species?

10. Early Development of the Chick

Original procedure: The objective is to introduce students to the post-gastrulation chick embryo. Students crack eggs containing live 3-day embryos and remove the embryos from the yolk. They observe the young chick's heart, blood flow, somites, and extraembryonic membranes. They also remove the amnion. Subsequently they use the dissecting microscope to identify many features of a fixed 33-hour embryo (central nervous system, blood vessels, heart, gut) and compare the features to those of a 48-hour embryo.

Group suggestions: Students should become invested in the lab's outcome during the week before the lab. For example, they could work in teams to create a concept map of all the factors that contribute to chick embryonic development: oxygen; humidity; hen's exposure to antibiotics, pesticides, or insecticides (or the same chemicals injected into the egg); hen's diet; egg incubation temperature; CO_2 levels, etc. Then, each team could decide on a treatment to investigate and design an experiment with a treated and control egg. The students could then investigate both the normal (control) embryo and how the parts change in the treated egg.

11. Muscle Contraction

Original procedure: This lab examines the contraction of skeletal muscle *in vitro*. The two experimental systems are (1) an actinomyosin gel made from homogenized rat skeletal muscle, and (2) sarcomeres from a rabbit muscle. Students observe contractions in both systems and design an experiment to determine the conditions required for contractility.

Group suggestions: This group did not develop a new procedure but rather pointed out that muscle contraction – the movement of actin filaments and myosin within cells – is just one of many examples of the cytoskeleton in action. Other examples of cell movements in various kingdoms include algae, amoebae, and *Tetrahymena* in kingdom Protista; hyphal tips in kingdom Fungi; chloroplast movement in plant cells such as those of *Chara*; neurotransmitter packets, endocytosis, melanophores/ chromatophores, and viral infection in kingdom Animalia. The group suggested starting with a demonstration, then instructing students to ask questions such as "How could we stop the cytoskeletal movement without killing the cell?" and "How might you start it again once stopped?"

Subsequent discussion focused on what students could manipulate and measure. For example, if it is possible to measure the extent of movement, students could measure the effects of temperature on the rate of cytoskeletal movement in different species. Or students could investigate the role of ATP, cations, and sodium pyrophosphate in cytoskeletal movement. Or the instructor could challenge students with a question such as "How would you determine the threshold?"

12. Invertebrate Diversity

Original procedure: Students "march" through eight phyla of animals, identifying structures in live, preserved, or dissected sponges, cnidarians, flatworms, nematodes, earthworms, snails, clams, grasshoppers, and echinoderms.

Group suggestions: The group suggested using one living representative from each phylum and having students compare and contrast the phyla in each of the following six categories: (1) symmetry, body form, and skeleton; (2) development & reproduction; (3) feeding and digestion; (4) circulation and respiration; (5) locomotion & muscular system; (6) ecology and habitat/niche. Since each student or group couldn't gather meaningful information about all of these categories, the group suggested that

students become "specialists," do outside research, and present their findings to each other. Or, instead of presentations, they could work in groups to generate lists of questions, and then ask each "specialist" group for the answers (cooperative learning). The instructor could reinforce this knowledge by providing unknowns. (The group noted that the instructor should emphasize that not all species in the same phylum share all features of the "representative" species). Another suggestion was to have students use characteristics they observe in unknown specimens to construct their own phylogenetic tree as a hypothesis to compare to the accepted evolutionary tree of life. The group also noted that similar approaches could be useful in labs covering plant diversity.

13. Plant Diversity

Original procedure: The two groups working on plant diversity considered a related group of labs entitled "Diversity of Life," "Mosses and Seedless Vascular Plants," "Lower Plants," "Seed Plants," "Fruits and Seeds," and "Structures of Flowering Plants." The labs all focus on the details of plant life cycles and morphology.

Group suggestions: The morning group focused on the adaptive explanations for student observations of plant diversity. For example, one theme might be how plants moved from water to increasingly dry environments. The lab could begin with an analogy, such as a video clip of animal reproduction (e.g. fish spawning, sea urchin egg fertilization, and mammals copulating on land). The instructor could provide an aquarium/terrarium with water, a waterfall, and a dry area to show the gradation of habitats. Students could then develop hypotheses (for example, about how different plants achieve fertilization) and make observations in that context.

The afternoon group mentioned two possible approaches that could be used separately or together. (1) Give students habitats to sample and answer the question "What grows there and why?" Have them name and describe as many different types of plants as they can, and describe how they reproduce. (2) Set up a diversity lab focusing on local plants. Direct students to solve problems (e.g. a game, scavenger hunt, or mystery) requiring them to identify groups. The lab could also tie in plant structure and relevance to student life, such as medicinal plants, indicator species (e.g. wetlands), and/or different parts used for food.

14. Soils

Original procedure: Students measure water retention in soil mixes with different amounts of organic matter. They also measure soil texture and composition (sand, silt, clay). Finally, students plant radish seeds in three soil types: pure clay, pure sand, and loam/humus mix. They then measure which soil yields the most plant growth after two weeks.

Group suggestions: Students could measure a variety of properties (e.g. physical properties, animal diversity, microbial diversity) in pairs of local soils collected from slightly different areas. For example, they might compare old growth to new growth, hilltop to valley, prairie to farmland, eroded to intact. Alternatively, they might compare different strata from the same soil, or investigate the effects of herbicides and pesticides on soil properties. If these "soil pairs" are unavailable, an instructor could have one or more colleagues ship soils from different parts of the country, and have students compare soils of different origins.

Conclusions

Working together, the workshop participants generated many ideas for transforming traditional cookbook labs to a more investigative format. As Crandall (1997) also noted, some procedures were easier to convert than others. But the groups found ways to make all the labs more engaging.

Most of the conversions shared common elements. Workshop participants tended to modify labs in such a way that students could be encouraged to manipulate the specific protocols in order to make the procedures stop, go faster, go slower, or become more efficient. This was accomplished in many of the labs through modification of standard or independent variables. Although many labs require that students understand specific procedures (e.g. use of a microscope or other equipment), students can learn these techniques either in the context of the investigative lab itself or in an earlier lab. Another common conversion technique was to present case studies, mysteries, or industry problems for students to answer or solve.

Investigative labs require some forethought, as instructors must provide plenty of possible "new" independent variables, creatively present a narrative or scenario, and keep up with multiple lab groups each doing slightly or greatly different procedures. But workshop attendees agreed that these might be minimized by instructors collaborating on ideas and requiring pre-labs and/or write-ups in which students present their hypotheses and procedures before the labs. Further, workshop attendees seemed to think that the extra work of making a lab investigative would be worthwhile in terms of student knowledge, learning, and increased scientific interest.

Several other investigators have published tools for converting cookbook labs into investigative or inquiry labs. For example, French and Russell (2001) published a "Lab Planning Worksheet" that helps instructors organize their ideas for concepts of interest, potential hypotheses, equipment, failure points, and pre-lab skills, concepts, and activities. Volkmann (2003) presents an approach that begins with an analysis of an existing lab and then provides 10 principles for adapting a lab to an inquiry approach.

No matter the exact approach used in converting traditional labs to a more interactive format, this major workshop session showed that these conversions are possible, to at least some extent, with virtually any lab. Also, the workshop participants frequently commented that interaction with other instructors greatly enhanced the quality of their suggestions for the "conversion." The result, we all hope, will be an increase in scientific interest and understanding among our students.

Acknowledgements

We gratefully acknowledge the dedication and enthusiasm of the participants in the two Conversion Immersion sessions. You were an inspiration!

- Enzymes (Laura DiCaprio, Christie Howard, Arthur Skura, Jan Simpkin)
- Osmosis (Laura DiCaprio, Christie Howard, Arthur Skura, Jan Simpkin)
- Fermentation (Mary Schaeffer, Amy Marion, Robert Grammer, and Jane Caldwell)
- Microscopy and Cells (Ann Lumsden, Anne Cordon, Norris Armstrong)
- DNA Isolation (Jenny Knight, Helene d'Entremont, Sonya Lawrence)
- Genetic Engineering: pGLO Transformation in Bacteria (Sue Karcher, Melody Neumann, Mark Walvoord)
- Antimicrobial Agents (Debby Filler, Bill Huddleston, Terry Ross, Maureen Waits)
- Effects of pH and Heavy Metals on Microbial Processes (Debby Filler, Bill Huddleston, Terry Ross, Maureen Waits)
- Immunohistochemistry (Morning: Jenny Knight, Helene d'Entremont, Sonya Lawrence; Afternoon: Sue Karcher, Melody Neumann, Mark Walvoord)
- Early Development of the Chick (Michelle Edgcomb, Michael Killian, Marshall Darley)
- Muscle Contraction (Seung Hong, Anne Cordon, Norm Scott, Mariëlle Hoefnagels)
- Invertebrate Diversity (Charlie Drewes, Saphida Migabo, Judy Guinan)
- Plant Diversity (Morning: Ralph Preszler, Heather Addy, Kelly Bohrer; Afternoon: Maggie Haag, Ann Lumsden, Dale Leady, Brenda Leady)
- Soils (Michelle Edgcomb, Michael Killian, Marshall Darley)

Literature Cited

- Crandall, G. Douglas. 1997. Old wine into new bottles: how traditional lab exercises can be converted into investigative ones. Journal of College Science Teaching. May 1997 issue, pp. 413-418.
- French, D. P. and Russell, C. P. 2001. Converting the Labs in an Introductory Biology Course from Cookbook to Investigative. National Association of Biology Teachers Convention, Montreal 8 November 2001. (Downloaded from http://zoology.okstate.edu/zoo_lrc/biol1114/guest/NABT-2001.htm).
- Volkmann, Mark J. and Sandra K. Abell. 2003. Tools for converting cookbook labs into inquiry. The Science Teacher. September 2003 issue, pp. 38-41.