With the rapid advancement of new discoveries in microbiology research, many instructors continually look for new teaching techniques that will give their undergraduate students a more fascinating and rewarding experience in the course. For this first volume of Great Ideas in Teaching Microbiology, Benjamin Cummings set out to collect “best practices” from educators who offered to share their teaching strategies with others. Whether you are a first-time instructor or a classroom veteran, we hope that this booklet will provide some valuable insights and methods that you can incorporate into your own microbiology courses. Please join us in thanking the educators who contributed their time and ideas to this project.

As a leading publisher of microbiology textbooks, lab manuals, and media products, Benjamin Cummings is committed to helping science educators reach their teaching goals. We invite all instructors to take advantage of our free Strategies for Success workshops, bi-annual Strategies for Success newsletters, annual Allied Health Student Scholarship Contest, and other services. We consider you and your students to be essential partners in the development of quality materials, and we invite your suggestions as we continue to improve our products and services.
Transitioning to Active Teaching and Learning

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Professional and Organizational Development Network in Higher Education (POD) Conference. Ten years ago, attending the first Undergraduate Microbiology Education Conference sponsored by the American Society for Microbiology changed my teaching forever.

Test the Waters Before Diving In

The only person who likes change is a wet baby. Some students will resist active learning. They view it as a waste of time and simply want you to tell them what they need to know to pass the course. Taking it slowly eases them into the process but, more importantly, it lets you decide what is going to work with your particular group of students.

Start with a low-risk technique, such as “Think-Pair-Share.” Look through your notes for the day’s lecture and decide where you might ask the student to provide a list. For example, on the first day of my general microbiology class, I display three images—a pond, a city, and a beach. I divide the students into three groups and ask each to take a mental field trip to the assigned location and think about the organisms that would be there. I then ask them to list at least ten of those organisms on a sheet of paper. It’s important to ask for a specific number as it keeps them on task for a longer period of time. Then I ask them to pair with a student who took the same field trip and group their 20 organisms into categories. Finally, I ask them to share their categories with the class. As I write their categories on the board, much discussion ensues and invariably a list of the Kingdoms of Life emerges. Yes, it takes longer than flashing a PowerPoint slide, but it begins the process of moving away from passive learning.

Begin with the Familiar

Active learning activities work best when the instructor is completely comfortable with the topic. Students can smell fear. I was formally trained as a clinical microbiologist and I teach nursing students, so I started with activities that took a clinical approach. Once I became more comfortable with giving control to the students, I was able to design in-class activities for other topics. An amazing thing happened! As the students were actively learning these topics with which I was less comfortable, I was actively learning right along with them.

As you begin transitioning to student-centered learning, try to incorporate at least one active learning technique during each lecture period. Look at your lecture notes and jot down some ideas in the margins. Where might you incorporate a Think-Pair-Share?
the students have already moved the chairs into group formation and are working on their latest project. The multiple choice tests and endless lectures have been replaced with group discussion, jigsawing, dynamic modeling, simulations, case-study analysis, and creative writing. This is exciting, and for this I'll gladly get out of bed for the next ten years.

**WEEKLY CONCEPT MAP ASSIGNMENTS**

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I teach a moderately large health science microbiology course (50–80 students). Many of the students are sophomore nursing majors with little or no background in biology or chemistry. I have tried several strategies to help students cope with what they often feel are vast amounts of material. The weekly concept map assignment has proved to be the most effective learning tool.

Each Monday I write 12–20 key terms on the board from a chapter to be covered in lecture that week. The assignment is to use all the words in a concept map, then write a summary explaining the map. The diagram may take any form as long as it shows how the terms relate to each other and to the main idea. Most students use a hierarchical flow chart or circle diagram with one central term and the others radiating out from it. I tell them the only unacceptable format is a list of definitions (which they could create by looking up all the words in the glossary instead of trying to understand their notes).

I go over a sample concept map on the first day of class so everyone knows what to expect. Most of my students haven’t done anything like it before and they have lots of questions about their first assignment. The first couple of maps are usually easy ones on the history of microbiology or microscopy applications.

Students who attend class regularly usually finish their concept maps quickly. By the time they have finished going over their lecture notes and organizing the terms into a logical diagram, they generally have a good understanding of the lecture material. Writing a summary of their map helps them process the information for long-term memory. Some students get quite carried away with their summaries, writing three or four pages of explanation; others just write down the words with a “goes to” or “includes” in between. Guess who does better on exams?

I encourage my students to work together on their concept maps, and many do. I suggest that if they do work together, they should write their own summaries, to see for themselves whether or not they really understand the concept. There are always one or two students who turn in a copied map and summary every week. As I always include at least one concept map on my exams, they find out that they didn’t learn anything that way. The concept map is usually worth 15–20% of the exam’s points, and the rest of the exam questions are computer-scored multiple choice. I can grade the exams fairly quickly because I can tell at a glance whether they understand the concept or are clueless. The few in the latter group often don’t even attempt a summary.

Weekly maps are due by 4 p.m. each Friday. I read them over the weekend and return them the following week. They are graded only with a checkmark, but I provide feedback by writing comments or questions if the map or explanation doesn’t make sense. Concept maps and lecture quizzes make up 10% of the course grade, or 100 points. (Lecture quizzes are short, unannounced open-book quizzes I give from time to time to encourage attendance. Some lecture quizzes are concept maps that we do in class.) The number of maps and quizzes varies each semester, but they usually end up being worth 4–5 points each.

**Sample Concept Map Assignment**

Draw a concept map using the words in the list below. Then write a paragraph summarizing your map—it should be clear from your summary that you understand the meaning and significance of each term on the list, and where it fits into the overall concept.

**Word list:** bacteriophage, conjugation, deletion, F pilus, frameshift, genotype changes, insertion, mutation, naked DNA, recombination, substitution, transduction, transformation

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**A Typical Concept Map**

**Benefits of Concept Mapping**

I have been using these concept maps for several years now, and my students say that doing the maps helps them learn. A common comment is that at first it seemed like busywork and a pain, but later they were glad they continued on page 4.
had to do them. From my point of view the benefits of this activity are how it:

- Enhances understanding of new material through active learning,
- Encourages students to keep up with lecture notes,
- Improves logical thinking and organizational skills,
- Is easier to grade than free-form essay questions.

I have found microbiology concept maps to be an effective learning aid for students and a valuable assessment tool for me.

**PATHOGENIC MICROBE PROJECT**

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This project is designed to replace or supplement the traditional lectures on pathogenic microbes in humans. There are several parts to the project, each designed to help students become familiar with a particular microbe in different ways.

A key goal is for students to become familiar with the signs and symptoms a particular microbe elicits in a human host, and how a physician/clinician might evaluate these to determine what the microbe is and how it should be treated. To do this, I have them write a case history, based on the format presented at the end of each chapter in their text (Tortora, et al.).

They also need to be aware of the wealth of information available beyond their text in Bergey’s Manual and other manuals for fungi and viruses. I have them write a summary of the entry for their particular microbe that describes its morphology, nutritional requirements, etc.

Another goal is for students to understand that the information in these manuals and texts is based on innumerable experiments done over the years, and is thus a dynamic, changing source. To help them realize this, I ask them to search out an original research paper in the primary literature reflecting research being done on pathogenic microbes and the diseases they cause.

Almost every profession that these students will have to do them. From my point of view the benefits of this activity are how it:

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Almost every profession that these students will enter requires teamwork. Working in groups (rather than individually) is more fun for some, and a real challenge for others. I encourage them to work on the whole project together, allowing individual efforts only under difficult circumstances. The final presentation is a poster, presented in class, which summarizes the case, the Bergey’s entry, the research article, and background material. I also request a picture of the microbe and three important characteristics of it. (I use the characteristics to form the stem of a matching test question for each microbe).

Following is the text of the Web page I use to direct students in the project. Underlined portions are links that:

- Lead them to their assigned microbe and group.
- Groups are based on lab groupings, which allows them to work on the project before, during, or after lab, and to discuss outside meeting places and times in the lab.
- Show a possible layout for the poster, allowing them to visualize all the parts they need to include and the points involved.

**The Pathogenic Microbe Project**

(Note: Your work will be graded by your group partners, your class peers, and by me.)

1. Each of you will be assigned to a group of three or four. This group will be assigned a pathogenic microbe. Individually, each of you should read the chapter in which the microbe and its effect on humans or other hosts is described. Then, you should write a brief case history of a particular clinical scenario starring this microbe. Model this case on those found at the end of Chapters 21–26 in the “Clinical Applications” sections. Your scenario should include details pertinent to the case, as well as two or three questions to help the reader. The case should be about one page long, including questions. Group effort is required. If this is a hardship for any of you, please let me know immediately. In your work as a group, please be sure that each member actually contributes. You will grade each other on this at the end.

If it is impossible to meet outside of class, the group should arrange a time when they can meet online in the MicroChat Room available on the Microbiology WebCT site. Each student should then submit a separate page with the case and questions.

2. The group will then find a recent research article on this particular organism in the library and submit the abstract to me for verification. Again, group activity is requested. Once you have the okay, briefly summarize this article in two pages.

3. Each student in the group should then read the description of the organism in question in Bergey’s Manual (or some other source if the microbe is not a bacterium, such as [list of resources in our library]), and submit a short two page description of the organism. This should include identifying characteristics, such as gram reaction, specific nutritional requirements, shape, etc., as well as a picture of the organism. (All of the above mentioned manuals can be found in the library.)

4. Finally, the group will organize a poster for presentation in class. The poster must include:
   a. A picture
   b. Your case and answers
   c. A one paragraph summary of your “Bergey’s” summary

GREAT (4) IDEAS
During the first week of class, each student is assigned to a team of four. One class period is devoted to announcing the teams, explaining the overall project, and setting up group meeting times outside of class. I pair four students based on past academic performance (although the teams appear as random to the students). A team consists of one high achiever, two average students, and one low achiever. This prevents the formation of teams that are academically unbalanced—that is, in need of a large amount of instructor assistance. Students with the highest past academic performance are assigned the role of group leader. Placing them in a leadership role gives them an opportunity to use their intelligence, good organization, and discipline to help the team succeed.

Each team draws an infectious disease (from a pool of diseases) to investigate. Diseases are typically those that have a dramatic disease course, or that have significantly impacted current or past cultures. Times for presentations are assigned with the disease so students will be able to plan and prepare for the project.

**Presentation Format**

The presentation is open-ended. Instructions are given only to frame the project and help students focus on essential content features that each presentation needs to communicate. To successfully complete the project students must:

- Develop a short, well organized, creative, clear presentation on the cause and effects of the assigned infectious disease.
- Work successfully with team members to develop the research topic into a presentation.
- Balance workload and responsibility among all team members.
- Construct a handout to effectively communicate the basic content requirements, and provide relevant new information about the disease.

There are several important constraints that make the project manageable:

- Each group constructs a class handout (under two pages) communicating key information about the disease that is distributed one week before the presentation. Information about the disease must minimally contain key features about etiology, transmission, pathogenesis, clinical features, diagnosis, treatment, and prevention.
- The oral presentations are strictly limited to 5–7 minutes. The time and page limits help students to focus on the essential features of the disease.

I emphasize that students need to involve each member of the team to devise a creative way to present the material so that other students will find it interesting. As a result, students are free to experiment with music, theater, art, computers, and multimedia to accomplish the task. Students take this task seriously;
The result is a collection of concise presentations that capture the interest and attention of the class.

**Findings and Benefits**

Since student presentations are the best part of the class, peers are almost never late. Absenteeism decreases significantly (an average of less than 5% per class, even in a large class of 100 students).

Presentations fall basically into three categories:

- Computer presentations that depend heavily on presentation graphics. This enables students to clearly organize the content of the presentation while taking advantage of photographs and animations to enhance the content.
- Skits that rely on the acting and writing skill of the participants to communicate information about the disease.
- Multifaceted presentations including video, skits and computer graphics. Individuals from hospitals, clinics, and law enforcement were recruited to add realism to the videos. Many students use comedy to draw on popular culture in creative ways.

Giving students a chance to present classroom material lets them express their creativity, humor, imagination, and dramatic skills. Although the presentations are at times outrageous and very funny, the disease features are still clearly presented. When groups focus on realism and the impact that infectious disease has on an individual or culture, their presentations are striking and effectively communicate the importance of control and prevention strategies to fight infectious disease. I was personally amazed at how much better the students communicated the information via these creative presentations than the traditional lecture format could.

**Active Science: Data, Analysis, and Library Skills**

**USING PRIMARY RESEARCH ARTICLES**

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One of my goals when teaching upper-level students is to have them read, understand, and think critically about primary research articles. Regardless of their chosen career path, students are confident with the application of concepts—and with themselves as scientists—after these exercises.

To begin the semester, students read and answer questions from several chapters of Microbe Hunters (DeKruif, P., Harcourt, 2002). This gives students information on the history of microbiology, helps them feel comfortable discussing material with their peers, and allows them to understand what is expected of them. For the rest of the semester, I select primary research articles that relate to a topic already covered in class (usually articles they can download in a format that preserves figures and tables). In the beginning, the entire class reads the same article; later, each group reads an individually assigned article. I instruct the students not to get bogged down with materials and methods (since they have not learned about or performed many of the techniques), but to focus on other parts of the article. Some techniques are introduced and explained when the article is distributed, and if possible, techniques are modeled or performed in a corresponding laboratory session.

The students work in their groups outside of class to answer questions about the article designed to help them understand it. Examples of questions include:

- What microbe did your paper use as its test organism and why?
- What was the problem being examined, and why is this problem important (big picture)?
- What were the results, and what do they mean?
- Do the charts and figures best illustrate the results?
- Did you need other sources to help you understand the article?
- Was the paper easy to understand using other sources and the knowledge gained in class?

Each group selects a “presenter” who is responsible for the overall explanation of the article. (Each student must present at least once during the semester). This aids students in discussing current scientific research and using scientific terms. I then facilitate a class discussion, which varies depending on the group of students and the difficulty of the article. I make sure each figure or table is discussed in reference to the data presented, other forms that the data could have been presented in, and the various statistical tests used (if any).

Many undergraduate (and graduate) students are reluctant to challenge published work. To assist them in evaluating the effectiveness and appropriateness of the research, I ask groups to give at least two criticisms of the research presented in their article. While students are initially reluctant, these critiques often spark interest, and let them know it is okay to disagree with the authors’ methods, results, and conclusions.
To determine their ability to analyze and convey information, my exams ask students to apply concepts from an article discussion to fictional graphs or figures. For example, after reviewing the Embden-Meyerhof pathway of glucose catabolism (glycolysis) and introducing other pathways during class lecture time, students read and discuss an article about pH regulation in citrate metabolism. Their exam then includes a graph or table similar to one found in the article—in this case, a graph that presents the effect of temperature instead of pH—and questions as follows.

**Example Exam Question**

You have decided to pursue microbiological research and have isolated a bacterial strain you call J. Koehl Strain SVC after your favorite microbiology professor. You are not sure of the importance of your microbe but you know it is a facultative anaerobe. You are characterizing glucose catabolism at varying temperatures (remember that glycolysis can happen by a variety of pathways). You obtain the results and present them in the following graphical form.

![Graph showing effect of temperature on the end products of glycolysis for J. Koehl Strain SVC.](image)

Answer the following questions regarding your results:

- Which glucose catabolism pathway(s) does J. Koehl Strain SVC use at each temperature? Explain your rationale.
- At which temperature does your bacterium grow worst? Why?

Questions such as these test not only understanding of the article but also the different pathways of glucose catabolism, a key concept in microbiology.

Overall, these exercises allow students to become comfortable with reading primary research articles, presenting scientific research, and discussing science with peers. In addition, they help students examine research results, scrutinize others’ research, and apply classroom concepts to the research being done.

**TEACHING STUDENTS TO WRITE SHORT DOCUMENTS FOR DIVERSE AUDIENCES**

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Each year when I teach Microbial Pathogenesis and the Immune Response, I incorporate several short writing assignments. As a liberal arts college professor, I strongly believe that we have the responsibility to teach future scientists how to communicate with the public. Therefore, in addition to the standard weekly one-page technical writing task, I also include a mock press release and a pamphlet.

**Three Kinds of Writing Assignments**

Teaching students to write should include writing for many different audiences. In my course, I have each student “adopt a pathogen” (for writing purposes only!). By the end of the first week, each student has signed up to write about a unique pathogen. Nearly every week thereafter, students turn in one page of technical writing about their pathogen pertaining to general topics that I present in lecture (e.g., physiology and growth, genetics, evolution and phylogeny, ecological niche, disease etiology, detection/treatment/prevention). Each assignment specifies information to include and asks for literature citations on a second page. By grading and returning these short assignments within a few days, I give students a chance to improve their writing, but I do not have to re-read the same document. By semester end, they each have written short, focused assignments with citations from the literature (which also discourages plagiarism).

At the end of the semester, two additional short assignments about their pathogens are due: a mock press release and a two-page handout (or double-sided tri-fold pamphlet) about the pathogen (and the disease it causes) that is suitable for third graders. The pamphlet is to have text, illustrations, and refer the reader to pertinent Web sites and print sources (encyclopedias and books of appropriate reading level.)

To assist me in grading these assignments, I often ask a member of the communications/public relations staff to rate the press releases. I have sought the cooperation of a local third grade teacher and her class to rate the pamphlets, so having some sort of relationship with the teacher or school beforehand is helpful.

**Press Release and Pamphlet**

To prepare for these assignments, students read official press releases from the public health department and visit local bookstores to see what reading level third graders should have. I also have several tasks that must be done in preparation.

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Teaching Students to Write for Diverse Audiences
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First, certain pathogens are not appropriate for third graders, e.g., sexually transmitted ones, or ones that end in horrific fatalities. For the students who have “adopted” those pathogens, I generate a different assignment for the pamphlet: why immunizations are important, why hand washing is important, or they can choose a disease that affects pets, houseplants, or crops.

I contact the third-grade teacher at the beginning of the spring semester. She or he can often work into the lesson plans ideas about good writing and how to offer constructive feedback. I compose a letter to the school principal and to the parents of students in the third grade classroom, giving my credentials and contact information, the purpose of the assignment, the timetable, and stating that participation is optional. I assure fears about nasty diseases, give examples of previous assignments, and names of other teachers to contact as references. These letters also go out early in the semester to give me time to answer any concerns. I have implemented this assignment three times and have never met with any problems from parents or administrators.

My students and I visit the classroom and throw a “thank you in advance” party for the third graders. We explain how important feedback and assessment are to college students. Students talk about their elementary school experiences, and relate to the third graders the importance of doing homework, turning it in on time, proper spelling, grammar, reading, etc. Since my students tend to be from diverse backgrounds, the third graders also learn important lessons about how different educational opportunities can be.

Assessment

To keep pamphlet grading anonymous, students turn in two cover pages (one of which has the authors deleted), as well as two copies of the pamphlet. I assign a number to each paper and keep the first cover page and the number to match up later.

Grading is done by the classroom teacher, using whatever rubric she and her class develop. These can include things that she is stressing in her own curriculum. I ask for a copy of the rubric and rating system. (I used to use their ratings directly as grades, but found that third graders are much too tough—they are willing to assign zeros, while I reserve zeros for missing assignments.) Rubrics have included the following (verbatim):

- Did not use words that we didn’t understand.
- Presentation was eye-catching (lots of color).
- Reader learned new information: “I’m smarter than my dad about this subject!”
- Reader was excited to learn more by checking a Web site (or library book) listed in the pamphlet.

The only thing I impose on the teacher is the deadline, as our semester ends about one month prior to theirs. I give the teacher no more than three weeks to return the pamphlets. I tentatively grade them myself, then consider the third graders’ ratings to reach the final grade. I found my grades to be swayed rather heavily by their feedback.

Outcomes and Lessons Learned

Initially, I did not treat this pamphlet as a very important assignment (in terms of percent of total grade). However, due to positive feedback from students, I “upped” the grade so that it is 20% of the total writing grade for the pathogen. (The press release is 10%.)

Students found the pamphlet the most difficult part of the total writing assignment. The most academically gifted students were often not the best at this type of writing. Those who were more creative and innovative were more successful. Other contributors for success were younger siblings or regular babysitting. If students babysat any of the third graders, they were never to discuss the assignment in any way. Students who read several children’s books in preparation for the assignment were also more successful. Several students, having completed the pamphlet, signed up to earn a teaching certificate. A few even chose to work toward a career as a science teacher, or an illustrator or author for children’s books about science and nature.

The third grade teacher enjoyed participating because it reinforced the writing standards she was teaching. We gave her extra credibility about the importance of sentence construction, spelling, grammar, etc. The third grade students saw themselves in a continuum of learning and were, often for the first time, the graders of an assignment. The importance of constructive feedback having already been emphasized, the teacher was subsequently motivated to add some peer-rev iewing to the third-grade assignments. As the instructor, I learned about developing rubrics and offering clear instructions at the beginning of the assignment.

The entire assignment was touted by the elementary school in a “highlights of the year” feature. Although some parents of second graders wanted to know if the assignment was to be repeated the next year, I have been
careful to distribute this experience over different schools and teachers. I do not want to see it become some sort of “privilege” or “burden” instead of an opportunity.

**Student Insights about Science Writing**

As a follow up with my own students, we discussed why this type of writing is important. Here is what they summarized:

- **Writing should be done by those with knowledge—it has the best chance of accuracy.** We gathered actual news clippings that inaccurately depicted aspects of health and disease. We discussed what the credentials of a science writer should be. Some students became interested in graduate science writing programs.

- **It is harder to write for people who have less experience/education, but it is often more important to share information with this type of audience than a highly trained technical group.** We talked about public posting, general instructions, package labels, etc.

- **Illustrations can be critical to communicating difficult ideas.** Students paid a great deal more attention to textbook figures than they did prior to completing this assignment.

- **Third graders can be really tough critics, and most of what they say improves the document.** We divided critiques into categories: style, format, content, aesthetics.

- **Writing a short document (or making a short speech) is much more difficult than writing a long one.** Having students keep a list of information they chose to leave out and why it was omitted was very useful.

- **Reading is the key to good writing.** Those who read more children’s books had higher ratings.

As with all other aspects of teaching, I learn more each time I construct and implement an assignment. One year, the pamphlets were done by pairs of students; and in two other years, they were authored by individual students. I think the pairs worked well and resulted in fewer pamphlets for the third grade teacher to rate.

I encourage others to adapt this assignment for their own purposes, but I do not recommend any grades lower than third, since the assignment should be taken seriously by students who do quite a bit of writing already. Should others be interested in obtaining templates for the letters to parents or school administrators, I would be happy to share them. I learned a great deal about my students, often uncovering a completely different facet, when I graded this assignment.

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**WHAT HAPPENED TO THE RAW DATA?**

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There is no denying the importance of technology and computer analysis of large data sets in training the next generation of biologists. Increased access to computer-controlled instrumentation allows more complex experiments to be introduced into the undergraduate laboratory. This has many benefits for teaching and learning, and allows students to be exposed to a variety of modern techniques. The use of highly automated instrumentation is considered a plus in recruiting students, and is frequently described as realistic preparation for future careers in biotechnology and biomedical research. Often lost in the seamless connection of data collection and computer data processing however, are the fundamental concepts being assumed by the software and the requirement that certain criteria must be met for that analysis to be valid.

The ease of pushing a button and obtaining pre-plotted data and statistical analysis of the best fit for a curve have created a new breed of students, “the R² equals 1” brigade of experimental overachievers. The automated data analysis creates a sense of assurance that whatever the software spits out is accurate beyond question. Students beam with confidence as they paste standard curves that go through zero with R² values of 0.999 in their lab notebooks. There is no need to check when the computer has provided a verified seal of approval.

To counter this effect, we use a simple two-part lab exercise to develop an ELISA for antibody to an infectious agent. The first part includes a simple dilution series with an enzyme-conjugated bacterial IgG-binding protein mixed with substrate in a microtiter plate. This part of the exercise focuses on the enzyme reporter step of the ELISA. The expected outcome is the generation of a colored product from the substrate that will demonstrate direct proportionality with both enzyme concentration and time. As substrate becomes limiting, this proportionality eventually will be lost. The computer analysis should demonstrate a linear relationship with a slope of 1 and, in a certain concentration range, show an R² value close to 1.00. Figure 1 is the data determined by a number of groups after a 15-minute incubation period.

Students are high fiving each other because they have achieved an R² value of 0.9997. (Who cares about significant figures?) We ask, “Are you surprised at the slope of the curve?” After a little refresher on the equation for a straight line, $y = mx + c$, students deduce that the slope of the curve is negative and 0.985. Is this what...
they expect? After forcing the students to reconsider their results, having them look at the plate and visually noting which wells have more color, they realize things are not what they seem. A careful review of the protocol indicates that they did not check the wavelength at which the ELISA reader collected the data. A re-read of the plate at 495 nm results in the data in Figure 2, a slope close to 1.00, and again an R² value of 0.9997.

The second part of the lab measures binding of different dilutions of antibody using microtiter plates coated with a model antigen. In the initial binding step, antibody-antigen complexes are formed and then unbound antibody is removed by washing. The residual antibody bound to the plate is detected using a reporter system in which the bacterial IgG-binding protein-enzyme conjugate is incubated with the immobilized antigen–antibody complexes on the plate. After an appropriate incubation period and a washing step, substrate is added and product generation is measured using an ELISA reader. The students are required to read absorbance 15, 30, and 60 minutes after substrate addition and determine how absorbance relates to the experimental variable of antibody concentration. The typical results obtained are shown in Figure 3.

The students, who recently discovered that you need to interpret your data in the context of the experimental design, are confused when they get graphs like the ones in Figure 3. The R² values are less than 0.9, the slopes vary with time, and the best fit line misses many of the data points. Students are perplexed. What did we do wrong this time?

It is time again to get the students to work out what is going on in each step of the experimental protocol. We ask them to identify what is being monitored in the assay and how. When it is clear that the quantity of antibody bound to the plate is what is being measured and that the enzyme-conjugated IgG-binding protein is acting as the reporter system, the experimental protocol becomes clearer.

The readout for the enzyme–substrate interaction parallels the experiment from the first part of the lab and shows the expected direct proportionality between product generation and time of incubation. Why is this proportionality not also observed for the antibody dilution series?

![Figure 1. Microtiter plate read at 590 nm.](image)

![Figure 2. The same microtiter plate read at 495 nm.](image)

![Figure 3. Binding of antibody to immobilized antigen in an ELISA using an IgG-binding protein-enzyme conjugate reporter.](image)
At this point, students need to appreciate that the antigen–antibody interactions will be determined by the affinity and concentration of the antibody. Additional considerations related to off-rates and equilibrium during washing steps can also be addressed. Similar considerations also apply to the interaction of the reporter and its binding to the antibody present in the antigen–antibody complexes bound to the microtiter plate. A consideration of what limits antibody binding and why one would expect the curve to be sigmoid should result from this thought process. The focus should ultimately be directed toward the linear portion of the curve—the only region from which quantitative data can be obtained. In addition, the reliability of spectrophotometers to measure absorbance in the >1.5 range should be discussed.

This two-part exercise illustrates the importance of understanding the principles of an experiment as well as the hazards of relying on a computer system to both collect and analyze/interpret data. Having students challenge the significance and reliability of machine-manipulated experimental data is an important learning objective that can be achieved in a number of ways. Students can be provided with blatantly ambiguous data, like Figure 1, and asked to critically evaluate its significance. Alternatively, laboratory reports can require an explanation of how computer-generated results were derived from the raw data. Critical thinking exercises and lecture time can also be devoted to manipulating raw data. The efficiency achieved with automated, high throughput technology does not negate the need for students to understand what happens between collection of the raw data and the instrument-generated report. Reminding students that “the smoking gun is always in the raw data,” is a good lesson for the era of increased automation and advanced technology.

**STUDENT-GENERATED DICHOTOMOUS KEYS FOR IDENTIFYING UNKNOWN BACTERIA**

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Teaching microbiology at a smaller school presents several challenges due to limited resources. Labs that ask students to determine the identity of unknown bacteria is one such challenge. We have a limited number of organisms available and a limited number of tests for students to do on their unknowns. To make it somewhat more interesting for them, I have my students develop their own dichotomous key before they begin to identify their unknowns. Students begin by developing a data bank through biochemical testing of various known bacteria, then use what they’ve learned to develop a dichotomous key. They spend the final few weeks identifying the unknowns.

**Step 1: Generating the Data Bank**

First, students spend two weeks generating a data bank for identifying their unknowns. They do traditional biochemical tests such as:

- Gelatin test,
- Tributryin, blood, mannitol, and MacKonkey agar tests,
- Phenol red lactose,
- Glucose and sucrose broths (with Durham tube),
- Catalase and oxidase tests,
- Triple Sugar Iron agar (TSI),
- SIM deeps,
- MRVP broths,
- Motility media,
- Urea slants, and
- Nitrate broth.

Students do all of these tests on all the bacteria that could be among the pool of unknowns later in the semester. In other words, they do not do just two or three archetypal bacteria for each test; they put every species of bacteria through every test. Each pair of students is assigned 2–4 species of bacteria to do all of the tests on. Each species of bacteria has to be tested by two pairs of students so that duplicate results are obtained. This requires a pool of about 16–20 species of bacteria, which is usually all that we have available to us. Then the students pool their results and generate their own data bank of results for each species of bacteria that could be their eventual unknown. We then compare the test results to Bergey’s Manual of Systemic Bacteriology and the lab manual we use in our course. Sometimes we will not find something to compare our results to in those two sources because the test is not usually done on all species of bacteria. If we do find something to compare our results to, we generally get the same result as the manuals. However, not every strain of every bacteria behaves according to Bergey’s Manual, so if we feel certain our test results are repeatable, we use our own result for our data bank and not the Bergey’s Manual result.

**Step 2: Developing the Dichotomous Key**

Next, students use their data banks to come up with a dichotomous key that will give a unique solution for each bacterial species. Students begin by dividing all the bacteria samples according to their gram positive or gram negative status. Then I give them a few hints, such as using shape as a defining characteristic, or checking Bergey’s Manual to see that certain tests like catalase and continued on page 12
oxidase tend to divide bacteria rather cleanly. (This depends on whether the tests are applied to Gram positives (catalase) or Gram negatives (oxidase), but I don’t tell them that much.) Figure 1 shows a representative student-generated dichotomous key that leads one to the identity of the given bacteria with the given tests.

**Step 3: Identifying Unknowns**

Students then spend two to three weeks identifying unknowns. I give them a mixed culture of bacteria so they must first separate the bacterial species by streaking for single-colony isolation. The species will be easily separable by color or Gram stain so the first step isn’t too hard. Then they must use their dichotomous keys to guide them on what tests to do in what order. Barring a contaminant, the students almost always successfully identify their two unknowns.

An advantage to my method is that it gives students more ownership of the project than do some other methods. Their development of a data bank of test results and dichotomous key before they test the unknowns makes them feel more involved and in control of the project. One disadvantage is that it is not as open-ended as those methods that give students the unknowns and have them use their lab manuals and Bergey’s *Manual* to guide them. If a wide range of bacteria are available and a wide range of tests are available, it makes sense to make the project as open-ended as possible. However, since we have only limited numbers of bacterial species and limited numbers of tests available, it makes sense to me to recognize and work within those limitations from the outset.

**USE OF INVESTIGATIVE PROJECTS IN AN INTRODUCTORY MICROBIOLOGY COURSE**

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When students perform a series of “cookbook” exercises that expose them to a variety of staining procedures, culture techniques, and identification strategies, they are not challenged to make independent decisions, or to interact with other students in developing research protocols. In my course, I use various experiments that challenge students to design their own experimental protocols, perform the experiments, and document their results in reports formatted as they would be for submission to a refereed journal. The first 3–4 weeks of the course are spent having students master the appropriate manipulative techniques essential in the “doing” of microbiology, then they take on the first of several investigative projects.

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**Figure 1. Actual Student Dichotomous Key**  This key was created by student Michael Cooper in 2001. Under our conditions at that time, there was no unique solution that would distinguish between *E. cloacae* and *E. aesogenes.*
Individual Investigations

Students are given a project to microbiologically characterize an "environment" of their own choosing. Environments that have been chosen include:

- a leafy plant surface,
- the ear of a pet dog,
- a seat in a sauna,
- a water fountain,
- the surface of a lab bench,
- the earpiece of a telephone, and many others.

Students must develop their own protocol that indicates the sampling technique, the type of medium to be used, and the incubation temperature. The identification of the isolated organisms is accomplished by examining cultural morphologies, by using the Gram stain, and by biochemical testing (using either standard tube methods or rapid strip or cupule methods). This project reinforces basic culture techniques, provides students with more practice with isolation techniques, description of colonial morphologies, and Gram staining. Students are genuinely more excited about the process because they have a personal interest in the eventual outcome of the experiment. This project involves 3–4 weeks of lab time.

Group Investigations

A second type of investigation is done as a group project. I provide the class with a list of 5–6 projects, have each student select one of their own interest, and group students accordingly. This type of approach assures that students do not group themselves. Examples of projects include:

- Verification that a cleaning agent "kills" 99.9% of bacteria.
- Testing the efficacy of contact lens cleaners.
- Survival of organisms on wooden vs. plastic cutting boards.
- Survival of organisms on washcloths or towels.
- Measuring the effectiveness of hand soaps.

Each group spends time during the lab developing an experimental protocol. I review the protocol and answer questions, but deliberately try not to write the protocol for them. At the end of the planning session, I reassemble the entire class and have the groups critique the others’ experimental protocols. Groups also identify all of the supplies and cultures that will be needed to conduct the experiment. Materials are prepared for each group so they can carry out the experiments in the next lab period. Students benefit from working in "real life" group situations where each of their inputs is valued.

Semester-Length Research Project

A third approach that I have employed with considerable success is the use of a semester-long n investiga-

tive laboratory project. This project is phased, requiring students to meet deadlines during the course of the term. The intent is to develop experiences that will be challenging to students, and will engage them in limited but realistic research projects.

Student pairs work on projects such as isolation of:

- A bacterial virus,
- An oligotroph,
- An organism smaller than 0.45 mm,
- A non-sporeforming thermophile, or
- An organism capable of degrading EDTA.

Students are given an initial reference and are required to identify other references, develop a research strategy, perform the appropriate experiments, and submit a paper in the format acceptable for a journal such as *Applied and Environmental Microbiology*.

Students blossom when exposed to these investigative approaches. They are genuinely excited about their work, are eager to cooperate with each other, and discuss their projects in a manner similar to the interplay that exists among researchers at a professional level. I feel that it is important to develop experiences that stimulate student curiosity and allow flexibility in the design of their laboratory experiments. Students who have experienced the investigative approaches have thrived later in graduate school and in their research careers. It is easy to discard the old "cookbook" approaches when one sees the level of stimulation, excitement, and satisfaction engendered by investigations.

**Hands-On Learning**

**HANDS-ON DNA MUTATIONS**

*Martha Sharron Jones, Catawba Valley Community College, sjones@cvcc.edu*

When you discuss the DNA concepts—mutation, replication, protein synthesis—with students in microbiology, do they seem to be dazed by the material rather than dazzled? Have many of your students been out of academia for a while? Are many of your students visual and tactile learners? Many of my microbiology students are nursing students and have been out of the academic world for many years. Simply lecturing to these students about DNA results in low test performance. For these reasons, I devised a hands-on presentation on DNA mutations. This exercise helps students to understand how changes in DNA (mutations) may or may not change the sequence of amino acids in the proteins that is the expression of the genetic information. *continued on page 14*
Hands-On DNA Mutations

Students work in groups of four. Each group is given an envelope with all the materials necessary to produce a strand of DNA and to complete protein synthesis.

Materials
Genetic Code chart Make a copy from your textbook and paste onto an index card.
Codon “reading frame” Cut index card in half lengthwise and label.
DNA 5′-3’ Cut index card in half and label.
mRNA 5′-3’ Use other half of index card for this. Label.
Anticodon-tRNA-aa Cut an index card in the shape of the letter “T”. Label top of “T” as anticodon, write tRNA on the stem of the T, and write AA (amino acid) at the end of the stem.
RNA polymerase Cut a large letter “R” from an index card. Label.
Ribosome Cut a card in a “derby hat” shape and label.
Amino acids Cut index cards into 16 pieces each. Label in these quantities:
SER - 6 LYS - 1 ILE - 3 MET - 3 ALA - 4
HIS - 2 VAL - 4 THR - 4 ARG - 6 ASN - 2
GLY - 4 GLU - 2 LEU - 6 PHE - 2 ASP - 2
TRY - 2 PRO - 4 CYS - 2 GLN - 2 STOP - 4
TRP - 1
Peptide bonds Cut chenille sticks into fourths and put seven in each kit.
Nucleotides Cut six index cards into eight 1.25 × 1.5” rectangles (for a total of 48).
• Label 12 “U” on one side and “T” on other.
• Label 12 “A”, 12 “G”, and 12 “C”.

Procedure
I give students a handout that lists key terms and provides a step-by-step set of instructions. These instructions are interspersed with brief explanations, terminology, and “self-check” questions. I talk them through the exercise so they can ask questions, then they repeat it with a different sequence of nitrogenous bases. An abbreviated version of the steps follows.

1. Using the T, A, C, and G cards, students make a linear sequence of nitrogenous bases found in DNA and label it DNA 3′-5′.
2. Using the DNA strand as template, they make a strand of mRNA using the appropriate nitrogenous bases for mRNA (the A, U, C, and G cards).
3. Translation is simulated next. Students position the ribosome to “read” the first triplet of nucleotides (codon) in the mRNA, and position the tRNA anticonodon above the codon to illustrate its role in sequencing the amino acids. Using the genetic code chart, students find the appropriate amino acid, put it in position, and advance the ribosome and tRNA to the next triplet. They then place a peptide bond between the amino acids in the resulting sequence.
4. Students may then delete or add a couple of nitrogenous bases on the DNA to simulate a mutation.
5. They make the corresponding alterations to mRNA, then “send it to the cytoplasm” where it can be read as in step 3. A new amino acid sequence is made.
6. Students then note whether changes did or did not occur in the sequence of amino acids, and whether a different or incomplete protein was formed. If changes did not occur, students are asked to consider why, which leads to the notion of redundancy in the genetic code. Discussion of the significance of changes in the amino acid sequence ensues.

While some students do experience a change in the amino acid sequence from the original, some do not. These observations and why changes may or may not occur are addressed. Students are very receptive to this exercise and their performance on test questions about this material is greatly improved.

Finally, as I walk through the classroom, I observe several positive outcomes of this exercise. I can immediately see if students are understanding this material and its application. Also, I can assess student cooperation and interaction, as students assist each other in the understanding of DNA mutations.

TEACHING DIAGNOSTIC STRATEGIES IN A CLINICAL MICROBIOLOGY COURSE

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At a time when infectious disease captures headlines almost daily, the education of technologists and other biomedical professionals must reflect transformative growth and technological change. Rather than ever-lengthening lists of tests and procedures, clinical testing can be taught as a system of strategic protocols that empower students to consider critically and employ protocols suitable to varied clinical situations and laboratory settings. The same pathogen may be encountered in a tertiary care hospital or in a field facility investigating bioterrorism threats. Technology used in these environments may range from classic cultivation and metabolic testing to microarray technology.

The Medical Laboratory Sciences Program (MLS) of Hunter College was founded in 1969 to prepare undergraduate majors for entry level careers in the diagnostic
and research laboratories of NYC, even as it provides the background for further educational goals. The two semester Clinical Microbiology (CM) sequence was designed to teach the characterization and identification of common agents of infectious disease, as well as an understanding of microbial pathogenesis. In the more than two decades I have taught the course there has evolved a tiered strategic approach covering the fundamentals of microbial structure and physiology, diagnosis of many bacterial, fungal and viral pathogens, and the tools to address emergent microorganisms and new technology. What follows is a summary of this approach.

The Clinical Microbiology Curriculum

The basics. Microorganisms are introduced using substantial chemical detail, especially of the components of the bacterial envelope. Once characterized, structures are revisited in their role in cellular physiology, virulence, and antigenicity. Students come to appreciate microbial features as tools for identification and as contributors to virulence. Other central concepts include logarithmic growth, the diversity of microbial (compared with mammalian) metabolism, horizontal genetic exchange, and selection, especially with regard to antimicrobial resistance.

Diagnostic strategies. Prepared with fundamental concepts and basic lab techniques, students are introduced sequentially to three broad approaches to diagnosis of infectious disease (A–C, below). Close correlation of theoretical and practical lessons is key to learning and is facilitated by a single instructor for lecture and lab. The pathways are summarized below and in the table (which includes specific examples).

A. Cultivation and characterization of microorganisms.

- **Cultivation** and identification of microorganisms, even as they are studied as contributors to virulence. The diagnostic strategy is learned in the context of the fundamental elements of antigen/antibody reactions:

<table>
<thead>
<tr>
<th>Diagnostic Strategy</th>
<th>Specific Techniques</th>
<th>Pathogen Types</th>
<th>Typical Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A.</strong> Cultivation and characterization of microorganisms.</td>
<td>Light microscopy (including direct specimen exam); cloning microorganisms; demonstration of structural, virulence, and metabolic features (traditional and rapid methods)</td>
<td>Fast growing bacteria and fungi.</td>
<td>Strept., Staph., Enterobacteriaceae, Candida, opportunistic molds, many others.</td>
</tr>
<tr>
<td><strong>81.</strong> Ag/Ab reactions using known immunoglobulins to identify pathogens.</td>
<td>Enhanced and direct agglutination; direct fluorescence; Q-Cellung, soluble antigen detection (immune precipitation); EUSA</td>
<td>Bacteria, viruses, yeasts, toxins.</td>
<td>Strep. pyogenes, Neisseria pathogens, Legionella, Cryptococcus neoformans.</td>
</tr>
<tr>
<td><strong>83.</strong> EØ resolution of known Ag to detect diagnostically significant antibody.</td>
<td>Electrophoretic resolution of complex antigens, detect w/ enzyme-linked immunoglobulins.</td>
<td>Viruses, non-or poorly cultivatable pathogens.</td>
<td>HIV, HSV.</td>
</tr>
<tr>
<td><strong>C.</strong> Genomic analysis (+/- amplification).</td>
<td>DNA/RNA probes (sometimes following PCR) using known sequences with reporter molecules, including in-situ tissue specimens.</td>
<td>Viruses, non-cultivable bacteria.</td>
<td>Chlamydia, Mycobacteria, Rickettsia, dimorphic fungi, viruses (CMV, HPV).</td>
</tr>
</tbody>
</table>

...continued on page 16
Teaching Diagnostic Strategies in Clinical Microbiology

continued from page 15

- an unknown component—the Ag being sought,
- a known (probe) antibody with specificity for the presumed Ag,
- a visualization system to detect the reaction (see table).

Sources of error begin to take on a more central role in discussion. Cross-reactivity of Ab, requirements for stringent testing conditions, and strain variations all require a more critical interpretation of results than does the classic approach—and they reinforce the role of quality control procedures.

B2. **Ag/Ab reactions using known antigen to detect patient immune status (serology).** The diagnosis of infection by the indirect route of detecting an immune response, while historic, presents a new challenge for students accustomed to focusing on the pathogen. The fundamental pattern of using a known Ag as a probe to seek a homologous (unknown) antibody in serum, and a system of detection, is similar to B1. However, the concept of titration of serum to detect significance is a key enhancement. The ability of patient Ab to neutralize biological activity of some Ags (e.g., hemagglutination inhibition) adds depth. Sources of error now include patient factors such as prior infections, immune disorders, and others in addition to technical flaws of Ag/Ab reactions.

B3. **Electrophoretic resolution of a known antigen to detect diagnostically significant antibody.** Separating the components of a microbial antigen such as HIV virus in order to detect and quantify (by immunoblotting) the patient’s antibody response illustrates the complexity of microbial antigens, and has numerous applications in the biomedical lab. In addition to the diagnostic data, students come to appreciate the dual role of viral components as functional units (e.g., reverse transcriptase of HIV) and antigenic determinants. The improved sensitivity for HIV diagnosis, compared with unresolved ELISA, reinforces error analysis. Protocols in which screening diagnoses are refined by sequential testing are important clinical lessons. Theoretical treatment in the CM course is followed by hands-on experience in Immunology.

C. **Biological “probe” approach to identification (genomic).** Single strand nucleic acids are used to detect diagnostically significant genome segments of pathogens in patient specimens. The transformative change in biomedicine brought about by molecular technology is well illustrated by DNA probe techniques in infectious disease diagnosis. In-situ tissue-based genetic probe analysis for viral diagnosis (CMV and HPV) gives students an empowering classroom experience, as well as hands-on insight into viral pathogenesis. Discussion of nucleic acid amplification prior to probe diagnosis (e.g., for Chlamydia) allows consideration of the errors due to even trace contaminants in specimens. Students have hands-on experience with PCR amplification in other MLS courses, and are prepared to appreciate the array-based technologies which increasingly will underlie biomedical analysis during their careers.

**Outcomes**

To prepare talented science students to enter the shortage-plagued medical lab community is clearly a value. Similarly, to encourage inner city, largely immigrant and minority students to pursue scientific careers brings great satisfaction. Nearly 1,000 MLS students have entered stable and challenging careers since our founding. The blurring of distinctions among clinical subspecialties, and between diagnostic and investigative labs further enhances the options of graduates who have a firm grasp of strategic diagnostic protocols.

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**ASM News Update**

**ASM’s 11th Annual Conference for Undergraduate Educators**

*Facilitating Student Learning in Diverse Environments*

May 21–23, 2004, Xavier University of Louisiana
New Orleans, LA

This conference seeks to improve undergraduate microbiology education by pairing science with the latest teaching reform initiatives. It offers a unique opportunity for microbiology educators to network, share research, and exchange solutions to pedagogical challenges. For program, housing and registration information visit [www.asmcue.org](http://www.asmue.org). ASM’s on-line collection of peer-reviewed resources for teaching and learning in undergraduate microbiology education is available at [www.microbelibrary.org](http://www.microbelibrary.org).

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