Title: What’s the Chemistry of it All?
An Exploration into the Recent Outbreak of E. Coli in Europe

Robin Ford
Palm Harbor University High School

Abstract
This action plan is designed to spark student interest by examining the real world example of the recent outbreak of E. coli in Germany and the potential new strain found there. Students will culture two strains of bacteria, extract the proteins from their two samples to run a PAGE-SDS gel electrophoresis, digest the proteins in the gel and finally send the samples to University of Florida’s Proteomics lab to run them through the MALDI-TOF mass spectrometer to find the molecular weights of their protein. After a few highly technical biotechnology labs, students will spend the rest of year investigating the chemistry behind this investigation. The students will build a portfolio beginning with the initial protein analysis, followed by a tie in for each unit of study correlating to the chemistry I course outline.

Rational
Students are always asking why they have to learn chemistry. This activity will explore a current and relevant topic (E. coli outbreaks) and relate it back to the simple chemical principles governing the experiments on the bacteria and substances. By integrating this biotechnology investigation, the students will engage in an interdisciplinary learning activity aimed at increasing their base of knowledge of basic chemical principles and the impact of emerging pathogens on their lives.

Description of Teaching Unit
The protein lab will occur at the end of the first semester unit. At the end of semester one, we will have covered molecules and polarity. The discussion of bacteria and proteins will bridge knowledge from biology (which the students should have taken the previous year) to the current year. We will review the first semester of chemistry after the initial lab with tie-ins to the chemistry we have learned through the year. We will continue to reference the lab throughout the remainder of the year. We will go back and review the scientific method and areas of chemistry and scientific research from the very first unit. We will continue to cover the main ideas from the course and see how closely related chemistry is to the science of biotechnology. Students will record all data and observations in a laboratory journal. This journal will be updated during each unit of study.

Overview of unit
1. Lecture on emerging pathogens: E. coli outbreak in Germany and discuss significance to possible new strain consisting of a PowerPoint presentation and a class discussion.
2. Students set up scientific journal: “What’s the Chemistry of it All?”
3. 3-4 day Proteomics Lab (all work in student notebooks/folders)
   • Culture bacteria (two strains) – may be done for the students
   • Day 1: Protein Extraction
   • Day 2: Run PAGE-SDS gel electrophoresis
   • Day 3-4: Protein separation and staining
   • Protein in-gel digestion (send to UF for MALDI-TOF MS analysis)
4. Discuss significance of results and relate back to E. coli
5. Get mass spec data back from UF
6. Continue on chemistry I course outline and insert section from each unit (see the table for areas of correlation)
<table>
<thead>
<tr>
<th>Unit of study</th>
<th>Correlation between content and proteomics lab</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1 - Intro to Chemistry</td>
<td>5 main areas of chemistry, scientific method, measuring</td>
<td>7 - Chemical Reactions</td>
<td>Enzyme reactions</td>
</tr>
<tr>
<td>2 - matter and change</td>
<td>physical vs. chemical change, classification of matter</td>
<td>8 - The Mole</td>
<td>Size of bacteria and viruses compared to size of atom</td>
</tr>
<tr>
<td>3 - Atomic structure</td>
<td>Isotopic Abundance and mass spec data</td>
<td>9 - Stoichiometry</td>
<td>Build on chemical reactions tie-in and look at mass relations</td>
</tr>
<tr>
<td>4 - Electrons &amp; Periodic Table</td>
<td>Light (laser) and Role of electrons (mass spec)</td>
<td>10 - Gases</td>
<td>Graham’s law of Effusion</td>
</tr>
<tr>
<td>5 - Ions and Ionic Compounds</td>
<td>Ionic charge, role of functional groups on proteins</td>
<td>11 - Thermochem</td>
<td>Denature proteins</td>
</tr>
<tr>
<td>6 - Molecules</td>
<td>Macromolecules, polarity</td>
<td>12 - Solutions</td>
<td>Buffers</td>
</tr>
</tbody>
</table>

**Learning Goals:** As a result of this unit the students should...

- Recognize the relevance of emerging pathogens to their lives
- Be able to accurately use the equipment (micropipettes, set up and run gel electrophoresis)
- Learn the basics of protein extraction and gel electrophoresis
- Interpret the results and be able to see difference in protein expression
- Apply the concepts learned throughout the chemistry I course to our investigation on emerging pathogens.
- Interpret data in many way as understanding of the underlying concepts grows and record in a science journal

**Data Collection techniques/Student Assessments**

Students will be given a pretest/posttest on how relevant they feel biotechnology and emerging pathogens are to their lives and to chemistry. Students will keep a portfolio of lab techniques and results. In this portfolio, students will also complete unit specific assignments (readings, calculations, activities etc.) throughout the year pulling critical pieces out from our initial investigation. This portfolio will be assessed at the end of the year.

**ICORE**

Students will complete a modified version of the Proteomics lab that was performed during the 2011 ICORE program. In addition, the locker program will be utilized for equipment and Dr. Chen’s lab will be needed for the mass spectrometry data.

**ICORE equipment locker**
- Protein Electrophoresis Locker
- Pipetting Locker
- Vortex
- Minicentrifuges
- Microtube Racks

**Literature Sited**
www.davidson.edu/academic/biology/courses/molbio/sdspage/sdspage.html

**Budget and Budget Justification**

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial sample</td>
<td>$20</td>
</tr>
<tr>
<td>Protein extraction buffer</td>
<td>$105.00</td>
</tr>
<tr>
<td>Comassie Blue</td>
<td>$84.44</td>
</tr>
<tr>
<td>ACN</td>
<td>ICORE?</td>
</tr>
<tr>
<td>Trypsin</td>
<td>$20</td>
</tr>
<tr>
<td>Extra micropipette tips</td>
<td>$15.00</td>
</tr>
<tr>
<td>Eppendorf tubes (pack of 100)</td>
<td>$23.00</td>
</tr>
<tr>
<td>Polyacrylimide gels</td>
<td>$92.50 package of 10</td>
</tr>
<tr>
<td>Micropipette Locker</td>
<td>ICORE</td>
</tr>
<tr>
<td>Proteomics Locker</td>
<td>ICORE</td>
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</table>

$359.94
**TITLE:** What’s the Chemistry of it All? An Exploration into the Recent Outbreak of E. Coli in Europe

Robin K. Ford
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**KEY QUESTION(S):** What is the chemistry (based on the first semester of chemistry I) involved or related to the protein extraction and analysis of microbes.

**SCIENCE SUBJECT:** Biology, Chemistry and Biotechnology

**GRADE AND ABILITY LEVEL:** 10-11th grade honor’s chemistry students

**SCIENCE CONCEPTS:** nature of chemistry, scientific method, measuring, physical vs. chemical changes, classification of matter

**OVERALL TIME ESTIMATE:** 1-2, 50 minute class period

**LEARNING STYLES:** visual, hands on, collaborative

**VOCABULARY:** chemistry, matter, scientific method, Hypothesis, measuring, dimensional analysis, significant digits, classification of matter, physical vs. chemical properties, physical changes, chemical changes

**LESSON SUMMARY:** This lesson will review some of the main concepts in the first semester of chemistry and following a 2-day protein extraction and gel electrophoresis. In the initial investigation, students will compare two strains of bacteria by observing physical differences and observing the differences in the gels. We will have discussed the recent E. Coli outbreak and will use this to model how scientist can identify differences in bacteria by looking for different proteins. We will then tie in what we have learned so far in chemistry to the science of biochemistry. The students will keep a notebook with their initial lab and each unit of study in chemistry will have a corresponding tie-in to the area of biotechnology and emerging pathogens.

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<td>scientific method, measuring, conversions</td>
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<td>2 - matter and change</td>
<td>physical vs. chemical change, classification of matter</td>
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</tbody>
</table>

**STUDENT LEARNING OBJECTIVES WITH STANDARDS:**

The student will be able to...

1. Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues. SC.912.L.16.10
2. Identify the parts of the scientific method SC.912.N.1.3, SC.912.N.1.4, SC.912.N.1.1
3. Determine the number of significant digits in a measurement and convert using dimensional analysis MA.912.S.1.2
4. Classify a sample of matter as being a pure substance or a mixture based on it physical properties. SC.912.P.8.4
5. Differentiate between physical and chemical changes in matter. SC.912.P.8.2
SC.912.N.1.3
Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.

SC.912.N.1.4
Identify sources of information and assess their reliability according to the strict standards of scientific investigation.

SC.912.N.1.6
Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.

MA.912.S.1.2
Determine appropriate and consistent standards of measurement for the data to be collected in a survey or experiment.

SC.912.P.8.4
Explore the scientific theory of atoms (also known as atomic theory) by describing the structure of atoms in terms of protons, neutrons and electrons, and differentiate among these particles in terms of their mass, electrical charges and locations within the atom.

SC.912.P.8.2
Differentiate between physical and chemical properties and physical and chemical changes of matter.

*MATERIALS:

**ESSENTIAL**: student folders with protein extraction lab data (will complete journal entries here), student notebooks from semester I chemistry

**SUPPLEMENTAL**: computers for research

BACKGROUND INFORMATION:
One of the reasons that some students do poorly in chemistry is because they do not see the relevance to their lives. I will present the topic of emerging pathogens and biotechnology to the students and then use the lab to go back and review some key concepts in chemistry. The students will have knowledge of first semester chemistry coming into this class. This lesson covers day one of our review (units 1 & 2) I will continue to review following a similar pattern (journal entries and small activities relating what we did in the lab with what we have covered/are covering in chemistry). I have included a brief summary of the content that was covered in class that relates to this particular lesson.

Chemistry is the study of matter and the changes it undergoes. During the first unit, we focus on the nature of science. The students use the scientific method as a way to understand how scientific questions are formed, how data is collected and analyzed. I also cover the metric system and conversions in the first unit. The students spend time measuring correctly (to the correct number of significant digits) and then using their measurements in calculations being sure to carry the significant digits through their measurement. Unit 2 is a look into matter. Specifically, how is matter classified? The students will decide if something is a pure substance or a mixture. They will then further classify them as heterogeneous or homogeneous (if a mixture) or as an element or compound (if a pure substance). Students will spend time deciding if looking at chemical or physical properties. Then they will examine changes that matter undergoes and decide if those changes are physical (meaning no new substances are formed) or if changes are chemical (meaning atoms have rearranged or a chemical reaction has occurred).

ADVANCE PREPARATION: The in-depth lab involving protein extraction and gel electrophoresis of two strains of bacteria.

*PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:
1. Give students pretest on the relevance to the lab. They will complete this on the computer now and at the end of the year. The questions will assess how relevant they felt the lab was to the work we do in a chemistry I class. (5 min)

    Rate the following 1-5 with a ‘1’ being **not at all** and a ‘5’ **very**

    1. Chemistry affects all aspects of my life
    2. The possibility of new/emerging pathogens is important my life
    3. The protein lab we completed relates to what we have learned so far in chemistry

2. Review the main ideas of the lab. Start off asking “What was the purpose of the lab?” (The big question is to answer: “Is the new European E. Coli outbreak caused from a new strain? To do this we have to compare the protein segments from 2 types of bacteria to look for differences) 5 min

3. At this point, have the students work in their journal and respond to a few scientific method questions as a way to review for unit one (all letters will be response entries in their student lab notebooks) 10 min

   a. If the first step in the scientific method was to observe, what did you observe first about the two strains. List 2 similarities and 2 differences. Feel free to use drawing or diagrams.
   b. Based on those differences, did you expect to be able to see differences in the gels after the experiment was run? Why or why not?

4. Next, we will discuss the results in class and I will ask students to cite sources of error and possible extensions to research. 5 min

5. I will then review metric conversion with a couple of metric conversion relating to the lab. 10 min

   a. We used 1.5 µL in the lab, how many L is that volume? Record answer with units and to the proper number of sig figs.
   b. A bacterial cell has an average diameter of 0.5 µm. If a culture 7.5 mm across, how many bacterial cells could line up across the diameter? Record answer with units and proper sig figs.

6. Unit 2 in chemistry deals with matter and change. I will focus on two main points as review: classification of matter and the difference between physical and chemical changes.

7. I will ask the students to work with a partner and complete the next 2 sections in their journal (the next two questions). I will give them about 5-10 minutes to complete this.

   a. What are 2 physical changes that took place in the lab? How do you know they are physical?
   b. Make a chart in your journal and find 5 substances used in the lab that were mixtures (then specify whether they were heterogeneous or homogeneous) and then 5 that would be classified as pure substances (then specify whether each of those were elements or compounds)

8. If time, I will have the students find a new partner and compare. They should then add at least one more physical and chemical change and add 2-3 more substances to be classified.

*ASSESSMENT SUGGESTIONS:* Describe specific assessments for EACH objective:

- **Obj 1:** Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.
  - Will be assessed through a pretest and posttest. The post test will be completed at the end of the year when the students have completed their folders/journals

- **Obj 2:** Identify the parts of the scientific method
  - Assessed through class discussion. I will also visually observe work/drawings in journals
- Obj 3: Determine the number of significant digits in a measurement and convert using dimensional analysis
  - Assessed through checking the answers to the conversion problems (including a check of units and significant digits)

- Obj 4: Classify a sample of matter as being a pure substance or a mixture based on its physical properties.
  - Assessed through peer review of the students’ classification charts and class discussion

- Obj 5: Differentiate between physical and chemical changes in matter.
  - Assessed through peer review of the student’s determination of physical vs. chemical changes and class discussion

**EXTENSIONS:**

**ACTIVITIES:** I will follow this lesson with similar activities to review the first semester of chemistry and then after each unit for the remainder of the year.

**RESOURCES/REFERENCES:**

Great link with information about elements
www.webelements.com

General chemistry help site
http://chemed.chem.purdue.edu/genchem/index.php

Extension for information on macromolecules
http://www.moleculardetective.org/News.html