

Teacher's Preparatory Guide

The Effects of Colloidal Silver on Microbial Growth: Investigating Snake Oil Science



Purpose: The purpose of this investigation is:

- 1) To use electrochemistry techniques to generate colloidal silver
- 2) To utilize the product of this method to determine if the growth of *Saccharomyces cerevisiae* and *Escherichia coli* can be inhibited.

Time required:

Preparation Time (Prior to Days 1 and 2)

1. At least 48-hours prior to Day 1 and Day 2 activities prepare and cure the Jell-O® “Practice Plates” and the nutrient agar Petri dishes. This protocol will take approximately 30-45 minutes to complete.
2. At least 24-hours prior to Day 2 activity generate the colloidal silver for the streaking of the microbial plates.

In-Class Time (Days 1-3)

1. At least 45-minutes of class time on Day 1 to discuss proper microbiology streaking techniques.
2. At least 45-minutes for the Day 2 activity, including the resuspension of the lyophilized bacteria, yeast activation, culture plate streaking, and disposal of contaminated equipment.
3. At least 30-minutes on Day 3 for discussion of experimental results (i.e., discussion questions) and the disposal of contaminated material.

Level:

Techniques, materials, and equipment are appropriate for high school life science and/or general science curricula.

Teacher Background:

Tired of the old standby “Penny Lab” when introducing students to the scientific method? Looking for an engaging way to incorporate the scientific method with real-world situations? Exploration of nanosilver or colloidal silver allows students to explore a cutting edge field of science. Are you in search of a quick, yet compelling, way to demonstrate to students the value of thinking like a scientist in a practical scenario? This activity uses the scientific method as the mechanism for testing the effectiveness of a familiar product, easily generated in a science classroom, and the validity of its advertisement claims.

Practicing science requires objectivity in experimental design, the recording of data, and the reporting of experimental findings. There have been many instances where scientific evidence has been either vehemently disputed or ignored completely (Galileo, Darwin’s Theory of Evolution, Mendel’s pea plant results, McClintock’s jumping genes) by members of the scientific community. Some examples of “bad science” have ended tragically as a result of poor science methods or the failure of scientists, working on behalf of laypeople, to act in good conscience and with the highest ethical standards. Examples of this include the 1981 Hyatt Regency catwalk collapse, the 2006 Ted Williams tunnel ceiling tile failure, lead-tainted paint in children’s toys, and the recent controversy surrounding the use of Lipitor® and its pitchman.

In this investigation, students will explore the validity of purported claims that colloidal silver, generated by means of direct electric current (DC), when added to nutrient agar and exposed to bacterial and fungal cultures is an effective method for inhibiting microbial growth. Is colloidal silver being touted as an antimicrobial agent, or is it truly the “silver bullet” in the defense against disease? This investigation has the potential to answer these provocative questions in a practical application of the scientific method as student investigators explore these claims, examine data, and revise hypotheses accordingly. In addition, an ancillary goal of this experiment is to demonstrate to students, the importance of being critical thinkers, equipped with practical knowledge to make smart consumer decisions. Students will not be fooled by snake oil science after this investigation!

Nanosilver or colloidal silver has gained popularity with the increase in antibiotic resistant bacteria and the search for “natural” cures for infections. For an interesting perspective on the history and uses of nanosilver visit:

http://www.nanobiotech.org/uploads/files/2011NCNCC_NanobioTrack_RosalindVolpe_TranslationalNanomedPanel.pdf and <http://www.silverinstitute.org/site/silver-in-technology/silver-in-nanotechnology/>.

Nanosilver is also referred to as colloidal silver and commonly consists of silver ions (~90%) and silver nanoparticles (~10%). For colloidal silver, silver ions are produced by electrolysis (Used in this activity) and are dispersed in the solution. Students should understand that a virus can be between 20-300nm while a bacterium may be up to 1,000nm in size. The ions and nanoparticles are much smaller than the microbes that are affected by their presence. You may want to have a discussion with your students about the benefits and pitfalls of nanosilver after the activity is completed. There are many concerned with the fate of the silver nanoparticles in the environment. A guide for such discussion can be found at:

http://www.nisenet.org/catalog/programs/nanosilver_breakthrough_or_biohazard.

Materials:

At Teacher Workstation

Prior to Day 1

(Prepare "Practice Plates" and Colloidal Silver)

- Laser pointer (for use by instructor only)
- 2 pkgs of clear Jell-O or Knox® Gelatine (what size?)
- Hot plate
- 12 disposable Petri dishes
- Parafilm® wax
- Silver electrodes
- 250mL beaker
- 9V battery and leads

Prior to Day 2

(Prepare Nutrient Agar Plates)

- 1L flask
- LB Nutrient Agar Powder
- Disposable, pre-sterilized 100 x 15mm Petri dishes (2 sleeves of 25)
- Hot plate
- 20mL colloidal silver

Day 1 *(Demonstrate Silver Colloid Formation)*

- Laser pointer (for use by instructor only)
- Hand held UV lamp

Day 2 *(Prepare Microbial Samples)*

- 1 pkg Baker's yeast
- Lyophilized *E. coli* (HB101 K-12 strain)
- Hot plate
- 250mL beaker
- 1 Tablespoon of sugar
- Micropipet with 250 μ L capacity

At Student Workstations

Day 1 *(12 stations)*

- 8 oz. cups (12)
- Glo Germ 8 oz. bottle
- Hand held UV long-wave lamp
- Disposable 10 μ L Inoculating loops

Day 2 *(8 stations)*

- Poured agar plates (1 LB/*E. coli*, 1 LB/*E. coli*/Ag, 1 LB/yeast, 1 LB/yeast/Ag)
- Permanent marker
- Disposable 10 μ L Inoculating loops
- 10% bleach in 250mL beaker

Advance Preparation For:

Day 1:

1. Prepare 12 “Practice Plates” with Jell-O® as described in the next section.
2. Obtain 12 plastic or Styrofoam cups and aliquot a small sample of Glo Germ™ Oil to each cup. In addition, provide each student workstation with 2 disposable inoculating loops, and a handheld UV lamp (you may choose to purchase one of these and keep it at the teacher workstation for the students groups to use when ready).
3. Make an overhead transparency of the “Proper Streaking Techniques” image to share with students before they perform Day 1 activity.

Day 2:

1. Prepare colloidal silver sample as described in the next section.
2. Prepare 32 nutrient agar dishes (8 LB/*E. coli*, 8 LB/*E. coli*/Ag, 8 LB/yeast, 8 LB/yeast/Ag) according to the procedure described in the next section.
3. Equip 8 student workstations prior to the experiment with one Petri dish of LB/*E. coli*, LB/ *E. coli*/Ag, LB/yeast, LB/yeast/Ag, a permanent marker, 4 disposable inoculating loops, and 10% bleach solution sample.
4. The teacher workstation should have lyophilized *E. coli*, a disposable needle tip pipet (Flinn Scientific catalog #FB1260), 1 Baker’s yeast package, hot plate, 1 cup of water, and 1 tablespoon of table sugar.

Day 3:

1. After examining the results, adhere to proper disposal procedures for contaminated material.
2. Copy results handout for distribution.
3. Make an overhead transparency of the handout and analysis questions for moderating class discussion of results and questions.

Safety Information

(NOTE: Refer to www.flinnsci.com/Documents/miscPDFs/SafetyContract.pdf for a copy of the specific lab safety contract used with this investigation).

- a. Always wear your safety goggles and lab coat when working in the laboratory;
- b. Food and/or drink are not permitted in the lab area;
- c. Adhere to proper safety techniques for handling microorganisms;
- d. Work under a well ventilated hood or equivalent when working with bleach;
- e. Wash all glassware thoroughly in order to eliminate cross contamination;
- f. Always dispose of liquid chemicals in properly labeled receptacles;
- g. Follow decontamination guidelines included in the procedure when disposing of microbial contaminated equipment; and
- h. Prior to exiting the lab area, utilize proper hand washing techniques.

Directions for the Activity

The following directions apply to one class of 24 students. Increase or decrease the amount of materials according to your class size and/or the number of classes participating in the activity.

At least 2 days prior to Day 1 activity

Prepare Jell-O® Practice Plates for Demonstrating Microbial Streaking

1. Prepare a batch of gelatin by adding 2 ½ cups of boiling water to 4 packets of Jell-O® or Knox® Gelatine. Stir the mixture, and let it cool before pouring into the practice dishes.
2. While cooling, label a class set of 12 Petri dishes “Practice Plates” (1 plate will be distributed to a team of 2 students during the in-class exercise). Do not allow the gelatin to solidify.
3. Once cooled, fill each Petri dish halfway with the gelatin. Replace the cover, stack the dishes upright, and store them in the refrigerator until solidified.

At least 1 day prior to Day 2 activity

Prepare Colloidal Silver

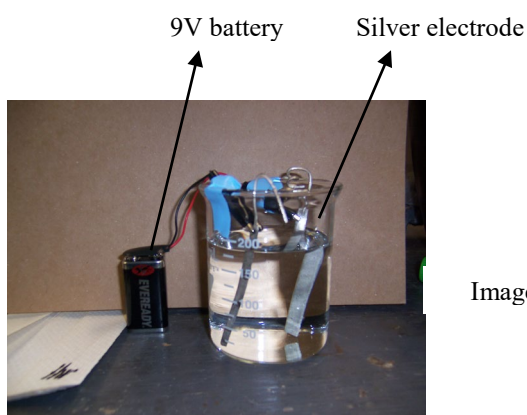


Image 2

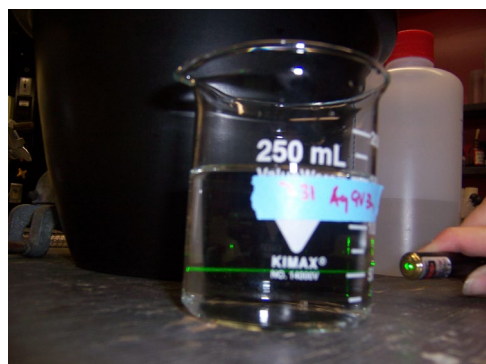


Image 3: Tyndall Effect

1. Colloidal silver can be generated by applying an electrical current through water using silver electrodes. Silver electrodes in the above pictures were made from Canada Maple leaf silver coins containing 99.99% silver (courtesy of Daniel Rosenberg, Lecture Demonstrations Associate, Harvard University). Silver coins can be obtained from on-line sources or local coin distributors. Silver coins containing less silver have not been tested in this protocol.
2. Setup colloidal silver “generator” as depicted in Image 2 above (explained in #3).
3. A clean 250mL beaker should be filled with 250mL of distilled water, and the silver electrodes should be placed a distance equal to the mouth of the beaker. Use the laser pointer to qualitatively determine the “purity” of the distilled water prior to attaching the electrodes

to the battery. In other words, if the laser beam is thick due to the scattering of monochromatic light, the sample is contaminated. If this is the case, wash and dry the beaker thoroughly, and repeat this test until the laser beam is thin and barely visible.

4. Attach leads from the 9V battery to the silver electrodes. Colloidal silver formation is indicated by the presence of bubbles in the water.
5. Allow silver to form in this manner for at least 3 hours. Avoid leaving the setup unattended overnight because mossy silver will build up on the surface of one of the silver electrodes because an alternating current is not being utilized.
6. Periodically check the status of the colloidal silver generated by using the laser pointer. A successfully generated sample of colloidal silver should result in a thick and vivid beam (see “Tyndall Effect” image above).
7. After the 3 hour period, dismantle the silver electrodes and 9V battery setup. Seal the 250mL beaker with Parafilm® wax to store overnight.

At least 24 hours in advance of Day 2 activity

A. Prepare LB agar

1. In a 1L Erlenmeyer flask, add the pre-measured packet of powdered LB agar (purchased from Bio-Rad catalog #166-0600EDU) to 500mL of distilled water.
2. By stirring/swirling, dissolve as much of the powdered agar as possible.
3. Heat on a hot plate until it has reached boiling. **CAUTION:** LB agar is extremely hot! Use hot gloves or similar for handling the flask and its contents.
4. Carefully monitor the LB agar as it boils. Do not allow mixture to overflow. A completed sample will appear clear when done.
5. When the sample has reached this stage, allow it to cool to approximately 50° C before pouring into pre-labeled, disposable 100 x 15mm Petri dishes (Flinn Scientific catalog #AP8170). DO NOT ADD anything to the mixture while it is cooling.

B. Label Petri dishes

1. While the Nutrient agar cools, use a permanent marker to label 8 plates LB/*E. coli*; label 8 plates LB/yeast; label 8 plates LB/*E. coli*/Ag; and label 8 plates LB/yeast/Ag.
2. There should be enough LB agar to make 32 plates, but you should make additional plates to replace dishes that may become contaminated.

C. Pour and store

1. When agar has sufficiently cooled, pour the contents into the Petri dishes labeled LB/*E. coli* and LB/yeast.
2. Then, to the remaining agar in the flask, add 20mL of the colloidal silver that was generated the previous night.
3. Swirl the contents of the flask to ensure adequate mixing. You may notice a slight change in the color of the nutrient agar from clear yellow to a clear dark yellow as a result of the addition of the silver. Once the colloidal silver has been combined with the remaining agar, pour the contents into the remaining Petri dishes labeled LB/*E. coli*/Ag and LB/yeast/Ag. Make sure that you do not overfill the dishes.
4. Stack the dishes, and allow them to cure for 36-48 hours in the upright position. After 2 days, invert the Petri dishes, and store them in the refrigerator until needed. Inverting the Petri dishes is not recommended for the dishes with silver because the concentration of

powdered agar to water, and therefore the stiffness of the nutrient agar, may have been altered as a result of adding 20mL of colloidal silver.

Day 1

Practicing Microbiology Techniques and Demonstrating Silver Colloid Production

1. Distribute one “Practice Plate” (Jell-O® dish) to each group of 2 students per student workstation. Also distribute, or have ready at student workstations, 2 disposable inoculating loops, a small sample of Glo Germ™ Oil (an 8 oz. bottle can be purchased from www.glogerm.com) in a paper cup, and a handheld UV long-wave lamp (Flinn Scientific catalog #AP1901).
2. Make a transparency copy of the Petri Dish streak below (an enlarged version is attached), and display it on your overhead projector.

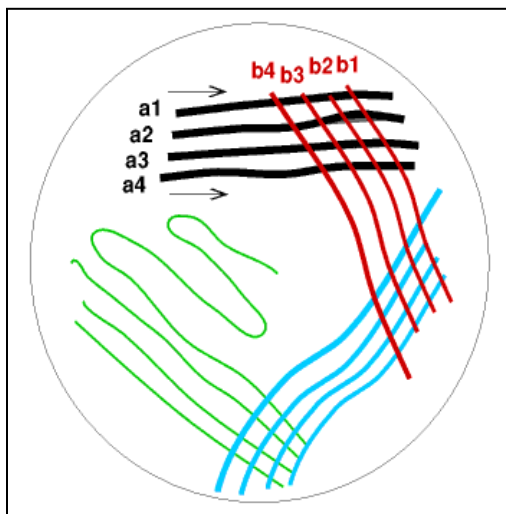


Image source: Courtesy www.biopics.co.uk/microbes/tech2.html

3. Simulate the process of streaking the Petri dish with the microbial sample using Glo Germ™ as the microbial sample, the inoculating loop as the instrument for streaking, and the Jell-O® “Practice Plate” in place of the nutrient agar plates.
4. Once students have practiced streaking their Jell-O® plates, have them use the hand held UV lamp to illuminate the streak lines they made on the “Practice Plates.” This approach is intended to familiarize students with the proper handling of instruments and microbial streaking techniques prior to the actual experiment.
5. **Optional:** If time permits, ask for a volunteer to smear the Glo Germ™ oil onto his/her hands. Then, use the UV lamp to show students how much the volunteer’s hands have been contaminated with “microbes.” Request that the volunteer wash his/her hands as s/he normally does, and then expose his/her hands to the UV lamp for a second time. The volunteer’s hands will *not* be clean as indicated by the orange glow under the UV lamp. Take this opportunity to stress the importance of sterile techniques and the safety precautions necessary when handling microbes. Show the proper hand washing technique on QuickTime video at www.glogerm.com to drive home the safe and hygienic practices, in the lab and at home.

Day 2

Streaking the plates

1. Each student workstation should have 4 cured Petri dishes labeled LB/*E. coli*, LB/yeast, LB/*E. coli*/Ag, and LB/yeast/Ag, 4 disposable inoculating loops, 10% bleach solution, and a permanent marker.
2. Resuspend lyophilized *E. coli* sample using distilled water per supplier instructions (Bio-Rad catalog #166-0408EDU). The following instructions are modified from the pGLO Teacher Manual developed by Bio-Rad (catalog # 166-0003EDU).
 - a. Add 250 µl of distilled water to the lyophilized bacteria sample. Let sit for 5 minutes and then shake vigorously at room temperature.
 - b. Use a 10 µl inoculating loop to remove a sample from the resuspended bacteria. Make sure there is a film of solution present across its diameter before proceeding.
 - c. Streak the Petri dishes by quarters using the method described above.
3. Once the bacteria have been prepared, use a sterile inoculating loop (obtained through BD™ Diagnostics catalog #219061) to streak the Petri dishes labeled LB/*E. coli*. Place used disposable inoculating loop into a 10% bleach solution for 20 minutes to decontaminate prior to disposal.
4. Repeat step 3 for the Petri dish labeled LB/*E. coli*/Ag.
5. Once the plates have been streaked according to the method described, and practiced by students, replace the cover and return the plates to the inverted position, use Parafilm® wax to wrap the stacked dishes. Store on the desktop for 24-48 hours for incubation at room temperature or in a 37°C incubator for 24 hours.
6. Similarly, prepare a sample of Baker's yeast according to the instructions on the package. This can be done while students are streaking the first Petri dish with *E. coli*, and should not exceed 10 minutes to setup. Alternatively, you may prepare the yeast prior to the start of class.
7. Proofing the yeast will determine whether the sample is active. This is accomplished by dissolving the contents of the yeast package in 1 cup (approximately 250mL) of warm water along with a tablespoon of sugar. If the sample bubbles, then the yeast is active.
8. Once activated, distribute a sample of the activated yeast mixture to each group and use a sterile inoculating loop to streak the remaining Petri dishes labeled LB/yeast and LB/yeast/Ag. Use a different inoculating loop for each dish and adhere to all safety practices for the handling of microorganisms, and the disposal of contaminated material. You can store these plates in the same way as the *E. coli* starter dishes and at the same temperature, and for the same duration.

Day 3

Analyze Results/Discuss Class Findings

1. Retrieve group Petri dishes labeled LB/*E. coli*, LB/*E. coli*/ Ag, LB/yeast, and LB/yeast/Ag.
2. Distribute the discussion sheet along with the accompanying diagram of Petri dishes and instruct students to record the results of the experiment.
3. **NOTE:** Both starter plates should be growing along the streak lines made during the previous activity. Cultured Petri dishes containing samples of *E. coli*/Ag and yeast/Ag may or may not show growth. If microbial growth is observable, draw the conclusion that colloidal silver at this concentration is ineffective at inhibiting microbial growth, and brainstorm follow-up experiments to test an alternative hypothesis. Conversely, if microbial growth is not observed (especially a result repeated by the majority of student groups), draw

the conclusion that silver at this concentration is effective at inhibiting microbial growth. Discuss the implications of this result for common online colloidal silver products. You may emphasize that colloidal silver may be effective at inhibiting the growth of these microorganisms, but may not inhibit the growth and proliferation of other species of microorganisms, including viruses.

Procedure (from [Student Activity Guide](#))

Day 1: Practicing Microbial Techniques

1. Obtain one “Practice Plate” of Jell-O® from your teacher. At your station, you should have 2 disposable inoculating loops, a small sample of Glo Germ™ Oil in a cup, and a handheld UV long-wave lamp.
2. Observe the process of streaking the culture dishes as demonstrated by your teacher. Simulate this process on your own Petri dish using the inoculating loops and the Glo Germ™ available. This approach is intended to familiarize you with the proper handling of instruments and microbial streaking techniques prior to the actual experiment you will perform tomorrow.
4. Use the hand held UV lamp to illuminate the streak lines that you made on the “Practice Plates.” How effective is your streaking technique?
5. Dispose of the materials as directed by your teacher, and wash your hands before leaving the class.

Day 2: Inoculating Petri Dishes

1. Your workstation should have 4 cured Petri dishes labeled LB/*E. coli*, LB/yeast, LB/*E. coli*/Ag, and LB/yeast/Ag, 4 disposable inoculating loops, 10% bleach solution, and a permanent marker.
2. Use the permanent marker to label your group name on the bottom of each Petri dish.
3. Once the bacteria have been prepared by your teacher, use a sterile inoculating loop to streak the Petri dish labeled LB/*E. coli* using the same method that you practiced yesterday. Place the used disposable inoculating loop into a 10% bleach solution for 20 minutes to decontaminate prior to disposal.
4. Repeat step 3 for the Petri dish labeled LB/*E. coli*/Ag.
5. Immediately replace the covers and return the plates to the inverted position. Use Parafilm® wax to wrap the stacked dishes. Store at your workstation, or other place indicated by your teacher, for an incubation period of 24-48 hours.
6. Your teacher will prepare a sample of Baker’s yeast. When the yeast have been activated, use a sterile inoculating loop to streak the remaining Petri dishes labeled LB/yeast and LB/yeast/Ag. Use a different inoculating loop for each dish and adhere to all safety practices for the handling of microorganisms, and the disposal of contaminated material. You can store these plates in the same way as the *E. coli* starter dishes and at the same temperature, and for the same duration.

Day 3: Interpretation of Results

1. Retrieve your Petri dishes labeled LB/*E. coli*, LB/*E. coli*/ Ag, LB/yeast, and LB/yeast/Ag. You will have an opportunity to interpret the results of your experiment.
2. Record your observations on the student handout distributed.
3. Discuss with your partner(s) the results of your experiment and work on the accompanying discussion questions. What do your results suggest about the relationship between colloidal

silver and microbes such as *E. coli* and yeast? What do your results indicate about colloidal silver as an antimicrobial agent? Are your results conclusive?





Cleanup:

All materials and equipment that come in contact with bacteria must soak in a 10% bleach solution for 20 minutes for decontamination. When decontaminating the Petri dishes, pour the 10% bleach into the culture dish, and let stand for 20 minutes. Excess fluid can then be poured down the drain. Collect decontaminated material, and double-bag the refuse for disposal in the normal trash bin.

Worksheet (with answers)

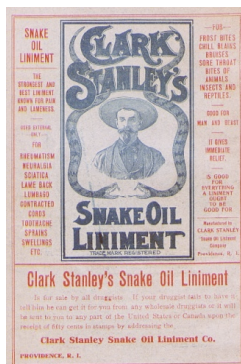
Name _____ Period _____ Date _____

Results of the Effects of Silver on Microbial Growth

	
<p>Petri Dish Name: LB/<i>E. coli</i> Control or Experiment? Control Record written interpretation of results: Growth should continue to grow with many, small to medium sized off-white colonies</p>	<p>Petri Dish Name: LB/yeast Control or Experiment? Control Record written interpretation of results: Growth of large off-white colonies similar to <i>E. coli</i> plate</p>
	
<p>Petri Dish Name: LB/<i>E. coli</i>/Ag Control or Experiment? Experiment Record written interpretation of results: Results have been inconclusive. However, further experimentation should show normal growth, similar to the control, at this level of Ag concentration.</p>	<p>Petri Dish Name: LB/yeast/Ag Control or Experiment? Experiment Record written interpretation of results: Results have been inconclusive. However, further experimentation should show normal growth, similar to the control, at this level of Ag concentration.</p>

Questions for Discussion

1. Describe the experimental design in this investigation. Identify the independent and dependent variables as well as the control(s) in this experiment.
2. Illustrate the culture dishes before and after the experiment on the handout provided. Identify the contents on each plate. Indicate which dish or dishes represent the control and which dish or dishes, if any, show growth.
3. Construct an advertisement similar the one below that promotes the benefits of colloidal silver.



4. On a recent family visit to see your great-aunt Lucy, you notice that the cough she complained about on your last visit has not responded to the cough syrup and regimen of lozenges that she keeps in her housecoat pocket. She tells you that her friends at Bingo were telling her about a novel therapy called “liquid silver” which is supposed to treat most major ailments that are bacterial or viral in nature. She is considering giving the treatment a try. Based on the results of this investigation and what you know about colloidal silver, what would you tell your great-aunt who is considering using a similar product to help subdue her persistent cough? Be persuasive.
5. Design a follow-up experiment based on your results. If your Petri dishes failed to grow, describe a controlled experiment to test why. If your Petri dishes successfully grew colonies of fungi and bacteria, describe a follow-up controlled experiment.
6. In 3 to 5 sentences, respond to the following statement: When a scientist has completed an experiment that supports his/her hypothesis, then his/her inquiry into this concept is complete.
7. **Extension/Enrichment:** Methylene blue (0.1%) stain is a common biological stain that can be used for determining the viability of yeast cells. Viable yeast pick up this dye and metabolize it while non-viable, non-metabolizing cells stain the color of the dye; in this case, blue. An operator, using a light microscope, can quantify the viability of yeast by withdrawing a sample from several hundred cells, exposing them to the stain for 5 minutes, and then calculating the ratio of stained cells to the total number counted. The results are then expressed as percent viability. This experiment may be repeated using yeast and *E. coli* samples that have been cultured in liquid nutrient broth (Bio Rad catalog #166-0421EDU) and subsequently exposed to a 0.1% methylene blue stain. Have students predict their results based on their results from the initial experiment. How do the results of a viability test compare to the results of the initial experiment that used agar cultures?

Assessment

1. Students will be assessed on the consistency of their results and the accuracy of their responses to the discussion questions (worksheet).

2. This lesson as it is can serve as an assessment, particularly if a lab report is assigned at the conclusion of the lesson/activity.
3. Students can demonstrate their understanding of microbiology techniques and snake oil science by presenting their information in pamphlets to an audience, or by integrating their findings as part of a larger project on disease or the immune system.

National Science Education Standards

High School Science as Inquiry

Standard A

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

Life Science

Content Standard C:

- As a result of their activities in grades 9-12, all students should develop understanding of:
The Behavior of Organisms

Acknowledgements:

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References

<http://www.bullioncoinsandbars.com/products-silver-coins.htm>

www.biotopics.co.uk/microbes/tech2.html

<http://www.nlm.nih.gov/exhibition/ephemera/medshow.html>