



Is the Gold in My Jewelry Real?

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Experimental Procedure

Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: [Project Involving Potentially Hazardous Biological Agents](http://www.sciencebuddies.org/science-fair-projects/competitions/biological-agents-regulations) (<http://www.sciencebuddies.org/science-fair-projects/competitions/biological-agents-regulations>) and [Scientific Review Committee](http://www.sciencebuddies.org/science-fair-projects/competitions/scientific-review-committee-src) (<http://www.sciencebuddies.org/science-fair-projects/competitions/scientific-review-committee-src>). You can also visit the webpage [ISEF Rules & Guidelines](https://www.societyforscience.org/isef/international-rules/) (<https://www.societyforscience.org/isef/international-rules/>) directly.

This science fair project involves the use of a classroom-safe strain of *E. coli*. While it is not considered a biohazardous or dangerous bacterial strain, it is important to always properly clean and dispose of bacteria and supplies that come in contact with it. See the [Bacterial Safety Guidelines](#) ([#bacterial-safety](#)) for more details on how to handle bacterial cleanup and waste.

Getting the Jewelry Ready

You will need to clean the jewelry before starting this science fair project. This will ensure that the surface of the jewelry is free from contaminating bacteria, and that the metal, gold, or otherwise, is in direct contact with the agar plates, rather than shielded by dust or oil.

1. While wearing disposable gloves, wash the jewelry in mild dish detergent with warm water. Rinse it well.
2. Gently dry the jewelry with a clean dish cloth and set it aside.

Preparing the Test Plates

1. Label four nutrient agar plates with the permanent marker.
 - a. Petri dishes should always be labeled in permanent marker and on the bottom of the plate. Labeling the lid is not sufficient, as the lid is removable and might accidentally get swapped with another plate.
 - b. One of the plates will be a control to make sure your bacterial lawns grow well and evenly. Label this plate: *Control*.
 - c. The other three plates will be your actual gold jewelry experiment plates—one for each piece of jewelry. Label them:
 - *Non-gold*
 - *Gold plated*
 - *Gold*, and the proper karatage. For example: *14-kt Gold*
2. To make your bacterial lawns, you will need to work in a sterile environment. Work next to a lighted Bunsen burner, but be careful around the flame. If you have long hair, tie it back. Do not wear flowing sleeves, and avoid reaching across the flame. If a Bunsen burner is not available, work next to a lit candle.
 - a. Take out your *E. coli* culture and follow the instructions in the kit to reconstitute the dried bacteria. Wait 5 minutes after reconstituting, then mix the gently shake to mix the bacterial suspension one more time.
 - b. Put 3 drops of the *E. coli* culture on the surface of an agar plate. Try to put the culture in the center of the plate. Use a sterile cotton swab to spread the bacteria around the entire surface of the agar plate. Put the lid on the plate as soon as you are done.
 - c. Repeat for the other three agar agar Refrigerate the remainder of the culture for repeats.
3. After you have spread *E. coli* on the agar plates, wait 5 minutes for the surface of the plates to dry. Remember, keep

the plates covered.

4. Now you are ready to place the jewelry on the plates.

a. Do not add anything to the *Control* plate.

b. Put on a fresh pair of disposable gloves. Add the corresponding piece of jewelry to each of the plates labeled: *Gold*, *Gold plated*, and *Non-gold*.

- Each piece of jewelry should be placed in the middle of the corresponding plate.
- Arrange each piece of jewelry in a manner so that the maximum amount of jewelry surface area is in contact with the agar plate. If the jewelry is made of flexible links, coil it so that it makes a continuous circle on the plate. See Figure 3, below, for a visual example.
- Try to make each piece of jewelry the same size and shape, preferably a circle, on the plate.



Figure 3. Each piece of jewelry should be arranged on its own plate. If possible, coil the jewelry to form a circle with the maximum amount of surface area in contact with the agar plate.

5. Re-cover and incubate the plates at 37°C for 48 hours. Make sure to invert the plates (lid-side down, agar-side up) so that any water condensation does not fall onto your bacterial lawn.

a. *Note:* If you do not have access to a 37°C incubator, you can grow the bacteria at room temperature. Keep the plates away from direct sunlight, but in a warm part of the house. For example, you may want to keep them in a plastic bag (to protect them from dust) next to a heating vent or the clothes dryer. The incubation time will be longer than in an incubator. Start checking your control plates after 72–96 hours of growing time.

Measuring Zones of Inhibition

1. After 48 hours of incubation (72–96 hours if you are not using a 37°C incubator), examine your plates (keep the lids on while you do this).

- a. Do you see a lawn of bacteria on the *Control* plate? If not, incubate the plates for an additional 24–48 hours until there is a decent bacterial lawn.
- b. The *Control* plate should show relatively uniform lawns. If you see dense bacterial growth in some areas and swatches of light or no bacterial growth in other areas, then your bacteria-spreading technique needs improvement. You will need to repeat the experiment again, paying special attention to spreading the *E. coli* culture across the plates to get reliable data.
- c. If the metal in the jewelry has an oligodynamic effect, you should see zones of inhibition around the jewelry.

The edges of the clear zone should be a relatively uniform distance away from the edges of the metal.

2. Using a ruler, measure, in millimeters (mm), the zones of inhibition.
 - a. If the three pieces of jewelry are all arranged in circles of similar size, measure the diameter of the clear zone.
 - b. If the jewelry is not circular, measure the distance from the edge of the clear zone to the first edge of the jewelry. Make several measurements around the piece of jewelry and average the measurements to get one representative data point.
 - c. Record all your data in a data table in your lab notebook.

Repeating the Experiment

1. To ensure that your observations are accurate and repeatable, carry out the experiment two more times.
 - a. If possible, use a new culture each time.
 - b. If a new culture is not available, remove the old *E. coli* culture from the refrigerator and gently shake tube. Leave tube at 37°C for one hour (or as close to that temperature as possible without exceeding it). Use this culture as your starting point for creating the bacterial lawn.

Analyzing the Data

1. Are the sizes of the zones of inhibition consistent across your replicates? Calculate the average and standard deviation for each piece of jewelry you tested. For more information about calculating and interpreting standard deviations consult the Science Buddies guide to [Variance and Standard Deviation](http://www.sciencebuddies.org/science-fair-projects/science-fair/variance-and-standard-deviation) (<http://www.sciencebuddies.org/science-fair-projects/science-fair/variance-and-standard-deviation>).
2. How do the average zones of inhibition compare for each type of jewelry? Did one type have a larger zone of inhibition than another? Do any of them show an oligodynamic effect?

Bacterial Safety

Bacteria are all around us in our daily lives and the vast majority of them are not harmful. However, for maximum safety, all bacterial cultures should always be treated as potential hazards. This means that proper handling, cleanup, and disposal are necessary. Below are a few important safety reminders.

- Keep your nose and mouth away from tubes, pipettes, or other tools that come in contact with bacterial cultures, in order to avoid ingesting or inhaling any bacteria.
- Make sure to wash your hands thoroughly after handling bacteria.
- **Proper Disposal of Bacterial Cultures**
 - Bacterial cultures, plates, and disposables that are used to manipulate the bacteria should be soaked in a 10% bleach solution (1 part bleach to 9 parts water) for 1–2 hours.
 - Use caution when handling the bleach, as it can ruin your clothes if spilled, and any disinfectant can be harmful if splashed in your eyes.
 - After bleach treatment is completed, these items can be placed in your normal household garbage.
- **Cleaning Your Work Area**
 - At the end of your experiment, use a disinfectant, such as 70% ethanol, a 10% bleach solution, or a commercial antibacterial kitchen/bath cleaning solution, to thoroughly clean any surfaces you have used.
 - Be aware of the possible hazards of disinfectants and use them carefully.