Which Acne Medication Can Really Zap That Zit?

https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p019/microbiology/acne-zits-medication (http://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p019/microbiology/acne-zits-medication)

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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) and Scientific Review Committee (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/) 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.

Preparing Plates for Acne Medication Effectiveness Test

1. Label your nutrient agar plates.
    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. Three of the plates are controls to make sure your bacterial lawns grow well and evenly. Label these plates: *Control #1, Control #2*, and *Control #3*.
    c. The other plates are your actual acne medication trial plates. Divide each plate into four equally sized quadrants with the permanent marker. See Figure 3 for an example.
        - Label one quadrant on each plate *Water*. This will be your negative control.
        - The other three quadrants should be labeled with the names of three *different* acne medications you are testing.
        - Each acne medication will be tested three times, but it should be tested on three different plates so that the maximum amount of experimental error is accounted for in your observations. Do *not* put all three replicates of an acne medication on the same plate.

        So, for every three acne medications you will label three plates where the quadrants read:

        A. Water, Acne medication 1A, Acne medication 2A, Acne medication 3A
        B. Water, Acne medication 1B, Acne medication 2B, Acne medication 3B
        C. Water, Acne medication 1C, Acne medication 2C, Acne medication 3C

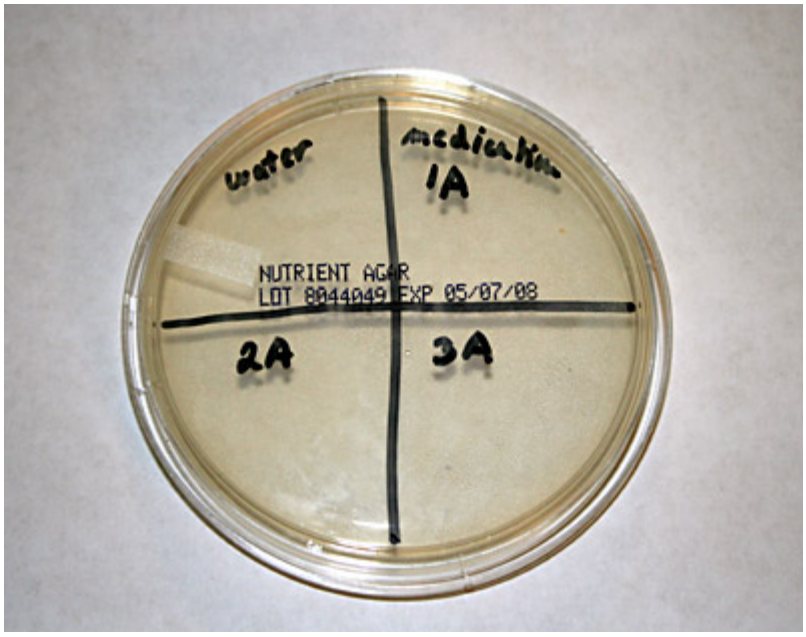

Figure 3. This photo shows how your agar plates should be divided into quadrants and labeled.

2. Boil a small amount of water in a pot for 5 minutes. After boiling, put a lid on the pot and let it cool to room temperature. This is your sterile water.
3. 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 2 drops *E. coli* culture on the surface of an agar plate. Try to put the culture in the center of the plate.
    c. 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.
    d. Repeat for the rest of the agar plates using a fresh cotton swab every two to three plates.
4. After you have spread all your agar plates, wait 5 minutes for the surface of the plates to dry. Remember, keep those plates covered!
5. Now you are ready to apply the negative control (sterile water) and medications to the agar plates.
    a. Do not add any substances to the three control plates.
    b. The remaining plates will each get a negative control—a disk dipped in sterile water—and up to three disks dipped in different acne medications.
        - Sterilize a pair of forceps or tweezers by dipping them in the isopropyl alcohol, then holding them in the Bunsen burner (or candle) flame for a few seconds. Let the metal cool for 1 minute before proceeding.
        - Using sterile forceps or tweezers, dip a sterile disk into the first acne medication you want to test. Gently remove shake or scrape off the extra medication without touching the disk.
        - Make sure that the label on the underside of the plate matches the medication you are testing. Place the disk in the middle of the appropriate quadrant on one of the agar plates. See Figure 4 for an example. Using the forceps, press down gently to ensure good contact between the agar plate and the disk.
        - Clean and re-sterilize the forceps between each use.
        - Repeat so that you have three plates for each acne medication.
        - Using the same technique, dip a disk in the sterile water. Apply one disk of sterile water to each agar plate. This will serve as a negative control. Sterile water should not create zones of inhibition; if zones do appear, you have a cross-contamination problem.

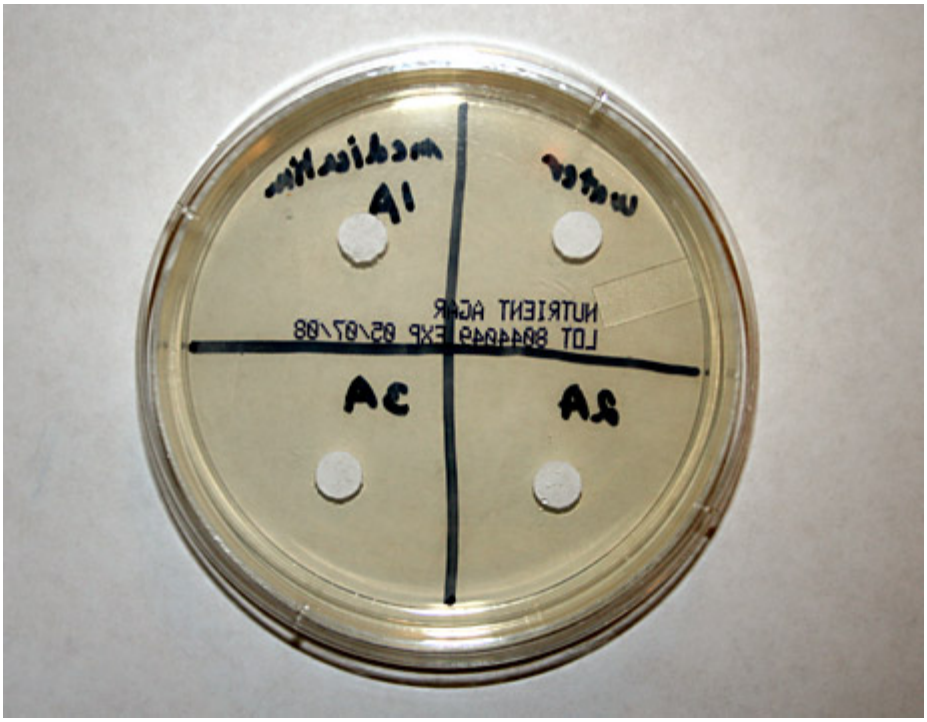

Figure 4. The experimental plates should look similar to this once you have applied the medicated disks to the agar.

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 three control plates you made? If not, incubate the plates for an additional 24-48 hours until there is a decent bacterial lawn.
    b. The three control plates 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 medications you chose are effective at preventing *E. coli* growth, you should see zones of inhibition around the medication disks. The clear zones around each disk should be circular with a relatively uniform diameter since diffusion of the substances through the agar is the same in every direction. If the zones of inhibition do not have uniform diameters, there are two possible problems:
        - The medication was not distributed evenly across the disk.
        - There was poor contact between the disk and the agar.
        - If the variability is particularly high, you might want to repeat the experiment, paying careful attention to how you apply the acne medications.
    d. There should be no zone of inhibition around your sterile water controls.
2. Using a ruler, measure, in millimeters (mm), the diameter of the zone of inhibition around each medication disk. Do not take the lids off to do this. Instead, invert the plates and measure with the agar side closest to you. Record all your data in a data table in your lab notebook. You will have three measurements for each medication, each from a different plate.
3. Analyze your data. Are the sizes of the zones of inhibition consistent across your replicates? Calculate the average and standard deviation for the medications you tested. Which acne medication was most effective; which was least effective?
4. If you cross-compare the active ingredients in each medication with the results of your experiment, do you see any trends? Are there certain ingredients that seem to contribute significantly to preventing *E. coli* growth? If so, consider designing an additional experiment to test your new hypothesis.

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.