



Experiment eBook





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Welcome and Thank You!

Thank you for purchasing our Prepoured Agar Plates and Bacteria Growing Kit! At EVVIVA Sciences, we are dedicated to making science fun and enjoyable, and we hope to encourage a passion and love for this field. Please enjoy this experimental eBook, which includes several fun and easy-to-do experiments you can perform using your kit. We truly appreciate your business and support, and we hope you love this product!

Disclaimer and Warnings

This kit is safe for all ages. However, some of the experiments in this eBook suggest the use of certain chemicals like bleach, which are toxic if ingested or if they come into contact with the skin. We recommend adult supervision when performing these experiments. Always wash hands thoroughly after performing a given experiment. Also avoid letting experiments go beyond 5 days as the bacteria and fungi can overgrow and contaminate their surroundings. Also, we try to verify all microorganism facts in this eBook, but not all have yet been verified, so please take some of these with a grain of salt 😊



Introduction and Basic Instructions

Our pre-poured bacteria agar plates are ready-to-use on arrival. The nutrient agar comes from an amino acid rich soy extract, which bacteria, yeast, and molds love! This kit is an excellent product for student science projects, and we hope you enjoy this complimentary experimental eBook for ideas. Plates are vacuum-sealed, completely sterile, and are not cracked or dried out. Our product is **100% SATISFACTION GUARANTEED**, and we hope you love it!

Includes:

- 10 pre-poured sterile TS (tryptic soy) nutrient agar plates
- 10 sterile cotton tipped swabs
- 1 bottle (0.5 oz) of anti-bacterial hand sanitizer to be used in experiments
- 2 Transfer pipettes

Basic Instructions

1. **Unopened plates can be stored up to one year** at room temperature or refrigerated (**NOT FROZEN**). Once the vacuum-seal is opened, plates should be refrigerated and used within 30 days.
2. **Take out bacterial agar plates** and let them warm up to room temperature for about 1 hour.
3. **Get a cotton tipped swab.** Wet the tip with water if you are going to be testing dry areas.
4. **Swab different objects** (teeth, phone, toilet, anything) to pick up microscopic bacteria and fungus.
5. **Gently wipe** the swab contents onto the surface of the agar plate.
6. **Close the lid and turn the plate upside down** so that the lid is on the bottom and agar is on the top.
7. **Incubate plates at 84-100°F** using a portable heater or a desk lamp. Keep a thermometer nearby to make sure the temperature is just right.
8. **Watch stuff grow!** Bacterial colonies or fungi should be visible in 1-4 days.



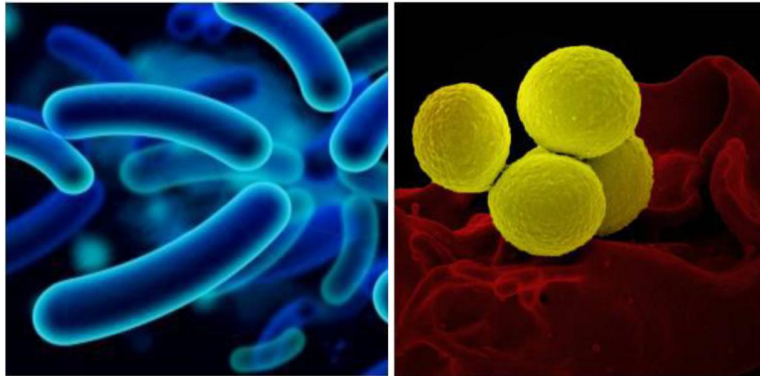
Top Tips!

1. **Keep plates at 84-100°F during your experiment.** Bacteria grow SLOWLY at low temperatures.
2. If plates are exposed to >100°F, **it can kill off bacteria**, and your experiment may not work.
3. **Be patient:**
 - Some organisms take more time to grow (up to 4 days).
 - If the object tested is clean and has very few bacteria, it may also take more time to get results.
5. **Do not use a blow dryer** to heat plates! This will cause plates to dry out.
6. **Do not put plates in the freezer** or the agar will crack when you take them out.
7. When swabbing a dry area, **wet the cotton tip with water** first! This helps to pick up more bacteria.
8. **Include a positive control.** Swab something with a lot of bacteria such as the inside of a toilet
9. **Have fun!**

Support and contact information:

If you have questions or concerns about your kit, please email us at support@evvivasciences.com, and a member of our staff will get back to you as soon as possible.

Bacteria and fungi: the good, the bad, and the fluorescent?



The world is filled with microscopic organisms (microorganisms or microbes) including bacteria, fungus, and other species. It is said that a single teaspoon of soil contains 1 billion bacteria, and there are more microorganisms on a person's hand than there are people on the planet! Many of these life forms have a bad reputation because some can cause diseases such as pneumonia, tuberculosis, athlete's foot, and strep throat. What many people do not realize is that most microbes do not cause disease (less than 5% do), and many of these organisms play important roles in our daily lives! Here are some interesting facts about microorganisms:



At least half of the oxygen we breathe is produced by microorganisms!



Many medications come from bacteria and fungi. Here are just a few examples:

- Botox, which is used to treat wrinkles, comes from the bacterium *Clostridium botulism*.
- Genetically engineered bacteria can be made to produce medications such as insulin and erythropoietin (EPO).
- Many antibiotics, which are medicines used to treat bacterial infections, are actually made by other bacteria and fungi. For example, Penicillin comes from the *Penicillium* fungus.



In the average household, it is said that the bathroom is cleaner than the kitchen. Test this for yourself in Experiment 1.



It is said that soap and water are more effective at killing germs than hand sanitizer. Test this for yourself in Experiment 4.



It was long thought that stomach ulcers were caused by stress and spicy foods, but we now know that they are actually caused by a bacterium called *Helicobacter pylori*.



Dr. Barry Marshall actually ate the contents of a petri dish containing *Helicobacter pylori* to prove that this bacterium causes ulcers. He indeed gave himself ulcers but he also won the Nobel Prize in Medicine for this discovery in 2005.



It is said that drying your hands with a paper towel after washing will reduce bacterial counts by 45 – 60%. However, it is also said that using a hand dryer may sometimes increase the bacteria on your hands by up to 255% because it blows out bacteria already living in the conveniently warm and moist environment. Test this in experiment 6.



Honey is a natural reservoir for the Botulism bacteria (the same bacteria that makes Botox). Adults can normally process it but infants often cannot, and this is why honey can be toxic to babies.



Treating patients with antibiotics may kill “good bacteria” in the intestines, leaving room for more dangerous bacteria to start growing. This can lead to a massive infection and inflammation of the intestines known as *Clostridium difficile* colitis.



Not all fungi are small. The largest living organism ever discovered is a honey mushroom in eastern Oregon, which spreads through the roots of trees and covers 2,200 acres (3.5 miles)! It is estimated to be over 2000 years old!



It is said that chocolate has an antibacterial effect in the mouth and protects against tooth decay. I’m not sure the dentist would agree with this, but you can test the effects of chocolate on bacteria using your kit.



When two people kiss, it said that they exchange between 10million and 1billion bacteria



Bacteria can be genetically engineered to express other animal’s genes. For example, fluorescent bacteria can be made from jellyfish genes. See figure 3!

Figures

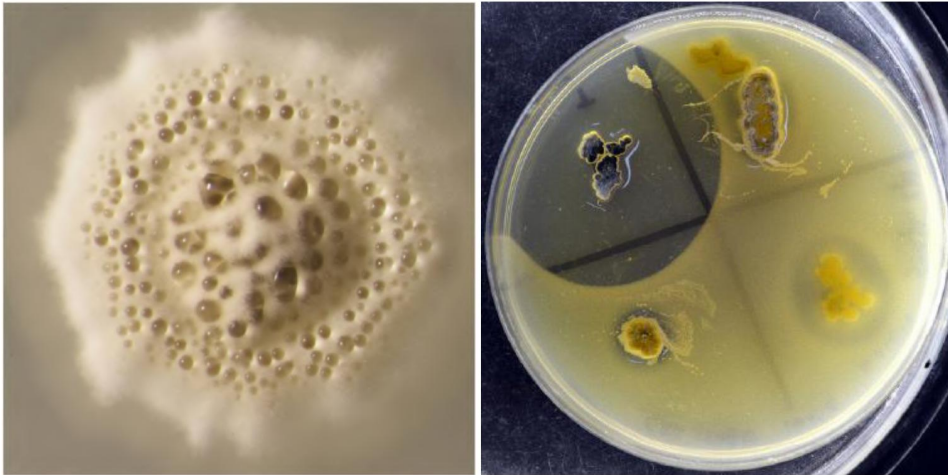


Figure 1: The good. The picture on the left shows a colony of actinomycetes bacteria, which produce antibiotics used to treat patients with infections. The picture on the right shows a petri dish with bacteria growth all over except in the upper left hand corner because of an antibiotic producing microorganism.

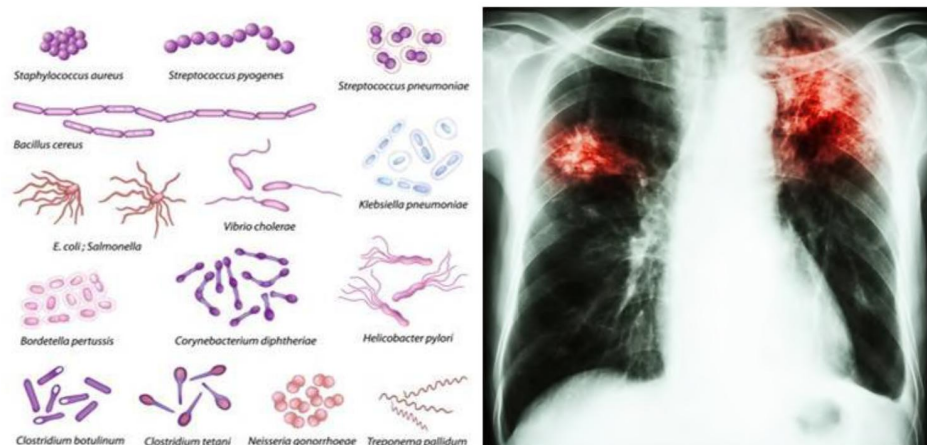


Figure 2: The bad. The picture on the left shows some of the types of bacteria that cause infections and disease in humans. The picture on the right is an x-ray of a patient with tuberculosis, an aggressive bacterial infection of the lungs. The red regions show the location of the tuberculosis bacteria in this patient's lungs.

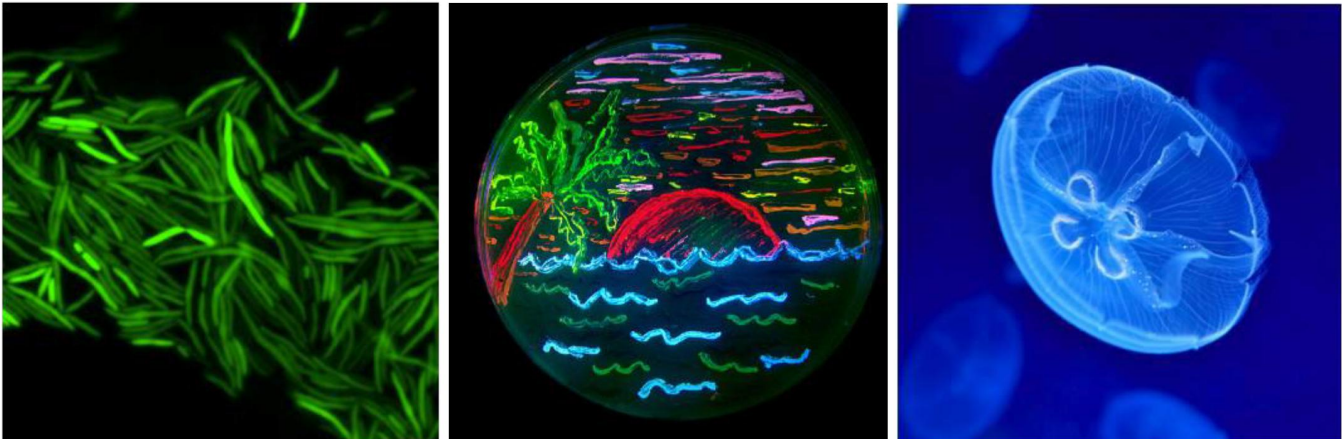


Figure 3: The Fluorescent. *Escherichia coli* or *E. coli*, a type of bacteria in our intestines, can be genetically engineered to express jellyfish (right picture) genes. The left picture shows *E. coli* engineered to express GFP (green fluorescent protein), a fluorescent protein produced by the jellyfish, *Aequorea victoria*. The middle picture shows a San Diego beach scene drawn with an eight-color palette of bacterial colonies expressing fluorescent proteins derived from GFP and the red-fluorescent coral protein dsRed.

Experiment 1: What object has the most bacteria?



This room looks pretty clean, but you will be amazed at what grows out on your agar petri plates!

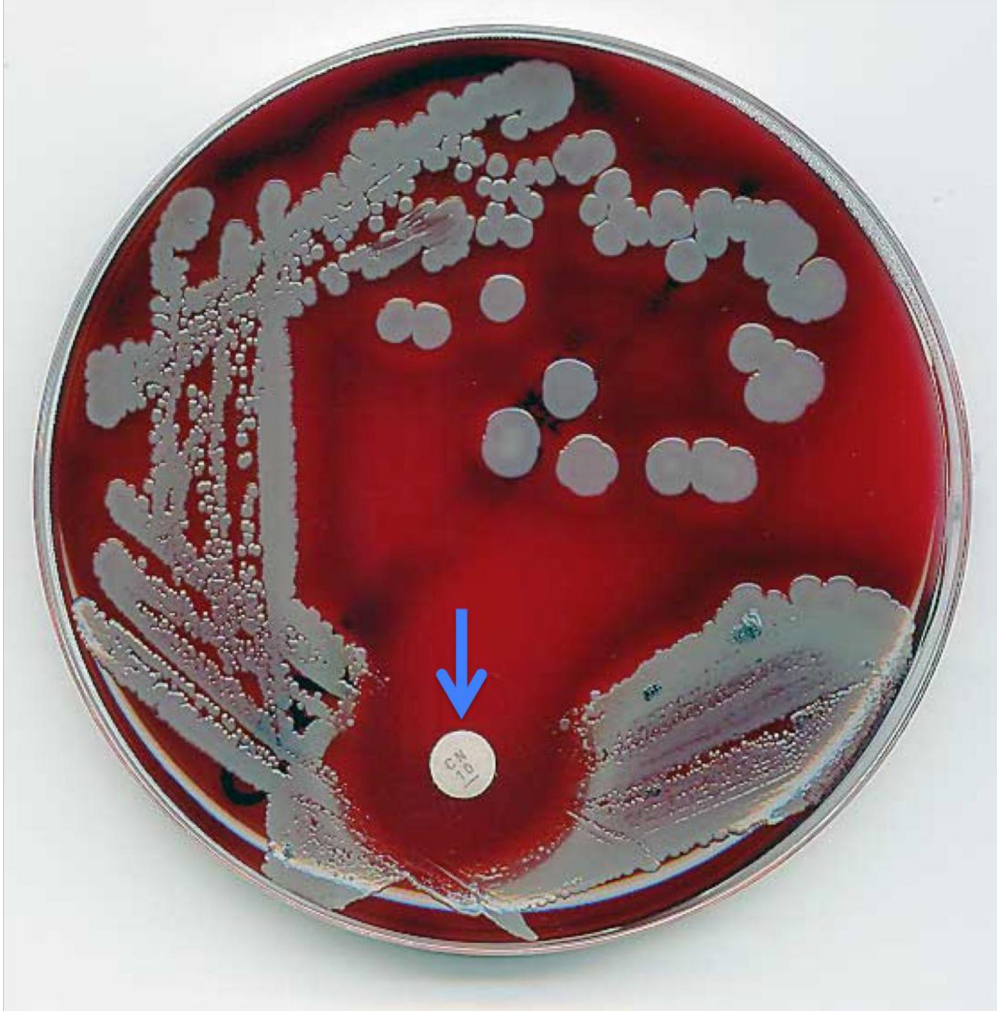
This is a classic experiment with bacterial agar plates. What has more bacteria, your cell phone or the toilet? Does a dog or person have more bacteria in his/her mouth? Is the kitchen sink cleaner than the bathroom floor? What part of your body has the most bacteria? Here is how you can test these and other questions:

1. Go around and swab different objects with the sterile cotton-tipped swabs included in your kit.
 - Use a separate swab for each different object you are going to test.
 - Remember to wet the cotton tip if you are swabbing dry objects!
2. Wipe the contents of each swab on a separate agar plate. You can also use a marker to divide plates in half to save materials.
3. Follow the basic instructions on page 4 (steps 6-8) to grow bacteria.



4. After several days, count the bacterial or fungal colonies on each plate to determine which object was the dirtiest!

Experiment 3: What is the best bacterial killer?



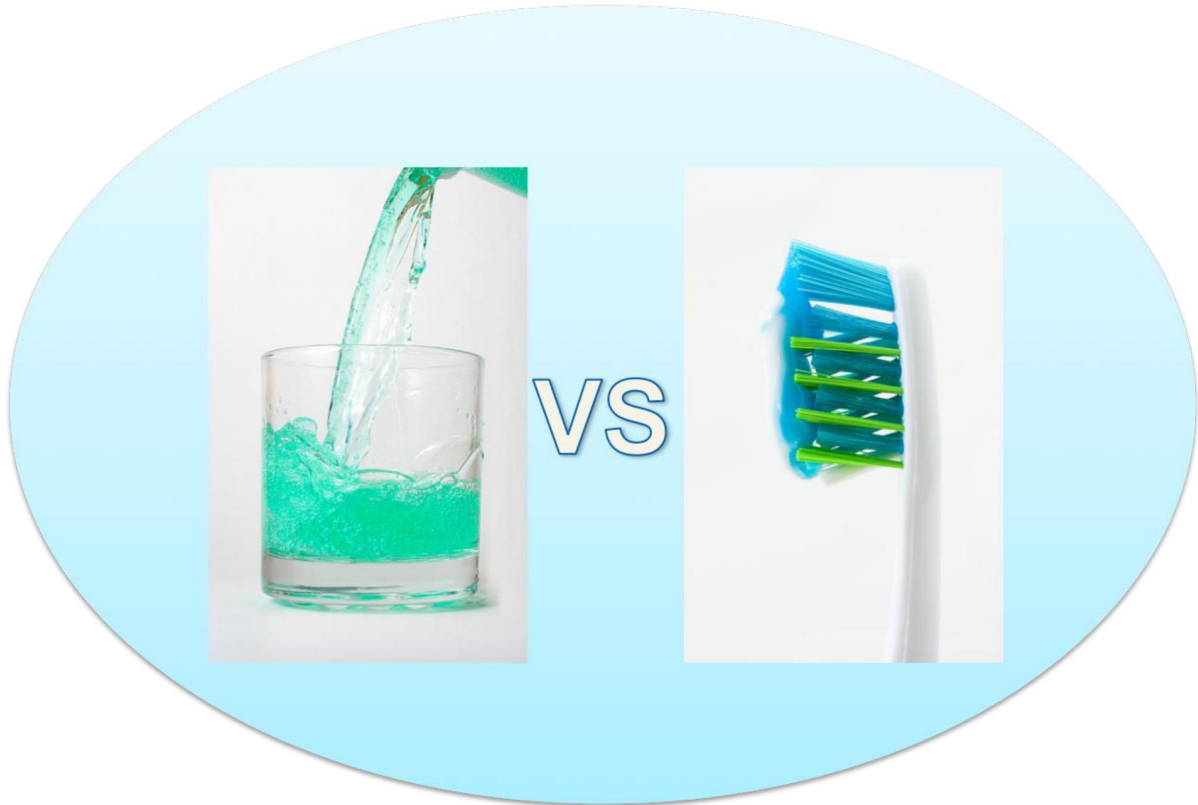
This picture shows a special type of agar plate, which contains a small amount of sheep's blood. That's right, it is pretty gross, but certain bacteria grow much better under these conditions. The gray areas are the bacteria. Notice that there is a white antibiotic disc near the bottom of the plate (blue arrow), which prevents bacteria from growing around it. In this experiment, you will create and test your own "antibacterial discs."

1. Use a sterile cotton-tipped swab on an object that has a lot of bacteria. It's gross, but the inside of a toilet bowl is a good place to try. Just make sure to wash your hands thoroughly after.



2. Wipe the contents of the swab onto one or several agar plates. Try to evenly distribute the bacteria across the plates as much as possible.
3. Now cut out several small paper-towel squares measuring about 0.5 inches x 0.5 inches.
4. Use a transfer pipette included in the kit to soak the squares in different antibacterial liquids. Try anything you want. You can try bleach, Lysol, hand sanitizer, soapy water, iodine, and any other liquids.
5. Include a paper towel square soaked with water as a control.
6. Place the wet paper towel squares on the agar surface of the petri plates that you had previously swabbed. Because the squares are moist, they should stick to the surface by themselves. You can include 3-4 squares for a single plate, but make sure they are separated from one another as much as possible.
7. Follow the basic instructions on page 4 (steps 6-8) to grow bacteria.
8. The paper towel squares should prevent bacteria from growing near them. Which antibacterial liquid worked the best? You can measure and compare the distances between the squares and the nearest bacterial colonies to help figure this out.

Experiment 3: Is toothpaste or mouthwash more effective at killing germs?



Wouldn't it be nice if you didn't have to brush your teeth? Well maybe not. But, what if mouthwash were just as effective at cleaning germs from your teeth as brushing them? Let's test this out!

1. Use a sterile cotton-tipped swab to wipe bacteria from your teeth. Wiping right near the gums is an excellent way to pick up bacteria! A great time to do this is right when you wake up in the morning before you've had a chance to brush your teeth.
2. Wipe the contents of the tip onto the agar surface of a petri dish. Label this plate 1.
3. Now brush your teeth for 1-2minutes and repeat steps 1 and 2 using a separate agar plate. Label this plate 2.



4. Grow bacteria on agar plates 1 and 2 using the basic instructions on page 4 (steps 6-8). Comparing plates 1 and 2 will show the effectiveness of brushing teeth on reducing germs in your mouth.
5. Wait until the same time the next day and repeat these steps but use mouthwash instead of brushing your teeth. You will have two new agar plates, one before mouthwash and one after mouthwash. Label these plates 3 and 4 respectively.
6. Begin growing bacteria on plates 3 and 4 using the basic instructions on page 4 (steps 6-8).
7. Grow each plate for the same amount of time. Find the ratio of bacterial colonies on plates 1 and 2 to determine how much brushing teeth reduces bacteria in your mouth. Now, determine the ratio of bacterial colonies on plates 3 and 4 to determine how effective mouthwash is at reducing bacteria in your mouth. Which one worked better?

Experiment 4: Soap and water versus hand sanitizer



VS



Hand sanitizers have become very popular but do they work better than old-fashioned hand washing? Let's find out!

1. Use a cotton-tipped swab on your hands before and after washing them with soap and water. Wipe the contents on two agar plates labeled 1 (before hand washing) and 2 (after hand washing).
2. At a later time, use a cotton tipped swab on your hands before and after cleaning them with the hand sanitizer included in your kit. Wipe the contents on two agar plates labeled 3 (before hand sanitizer) and 4 (after hand sanitizer).
3. Grow bacteria on plates using the basic instructions on page 4 (steps 6-8).



4. Compare which worked better at reducing bacteria on your hands; soap and water or hand sanitizer.

Experiment 5: What is the best bacterial killer part #2



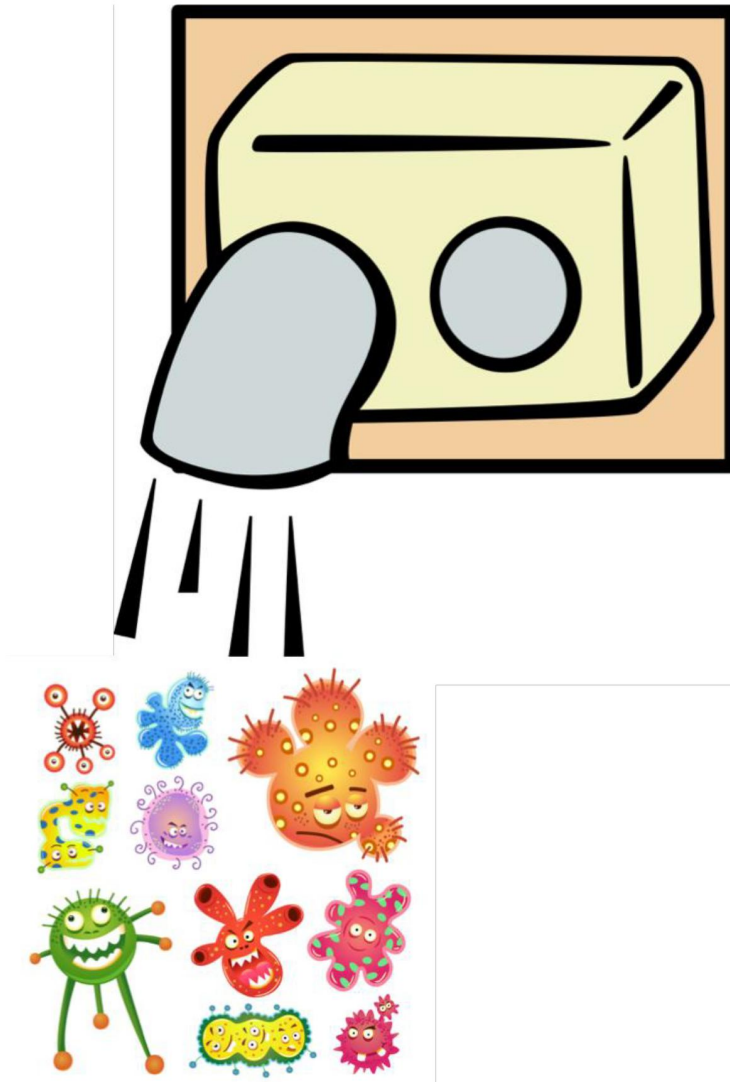
Here is another experiment to test which substance is best at killing bacteria. Enjoy!

1. Pour several 1/2-cup (4oz) glasses of milk.
2. Use the transfer pipettes in your kit to add different antibacterial liquids to each glass. For example, you can add bleach, ethanol, hand sanitizer, soapy water, Lysol, etc. We recommend adding at least 5-10ml per glass, and remember to add the same volume to each. Mix the glasses of milk thoroughly.
3. As a control, include one glass of milk without any antibacterial liquid added (or with plain water added).
4. Leave milk out on the counter for several days, which will give the bacteria in the milk time to grow. By this time, the milk will likely have curdled due to pH changes from these bacteria.



5. Dip cotton tipped swabs into each glass of milk and wipe the contents onto separate agar plates.
6. Use the basic instructions on page 4 (steps 6-8) to let bacteria grow
7. After several days, count bacterial colonies to determine which milk was the least "spoiled." That is, which antibacterial liquid protected the milk best from growing bacteria?

Experiment 6: Drying your hands with a paper towel or a hand blower



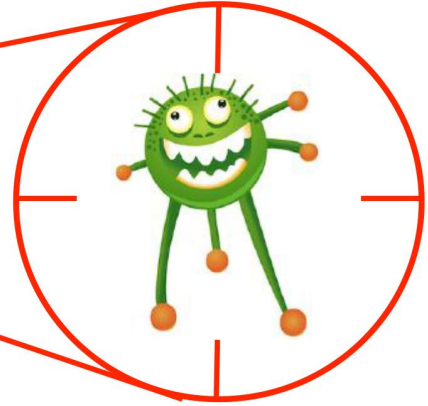
It has been said that using a hand dryer after washing your hands may actually increase the amount of bacteria on your hands because it will blow bacteria out onto your skin. Is this really true? Let's test it out!

1. Use a cotton-tipped swab to transfer bacteria from your hands to an agar plate. Label this plate 1.



2. Next wash your hands with soap and water for 30 seconds to 1 minute. Dry hands with a paper towel and use a cotton tipped swab to transfer any remaining bacteria from your hands to a separate agar plate. Label this plate 2.
3. At a later time, repeat steps 1 and 2, but use a hand dryer in a public restroom to dry your hands. Label these plates 3 and 4.
4. Grow bacteria using the basic instructions on page 4 (steps 6-8).
5. After several days, count bacterial colonies to determine whether using a paper towel versus a hand dryer was more effective at reducing bacteria on your hands. You can also try this experiment with just letting your hands air dry.

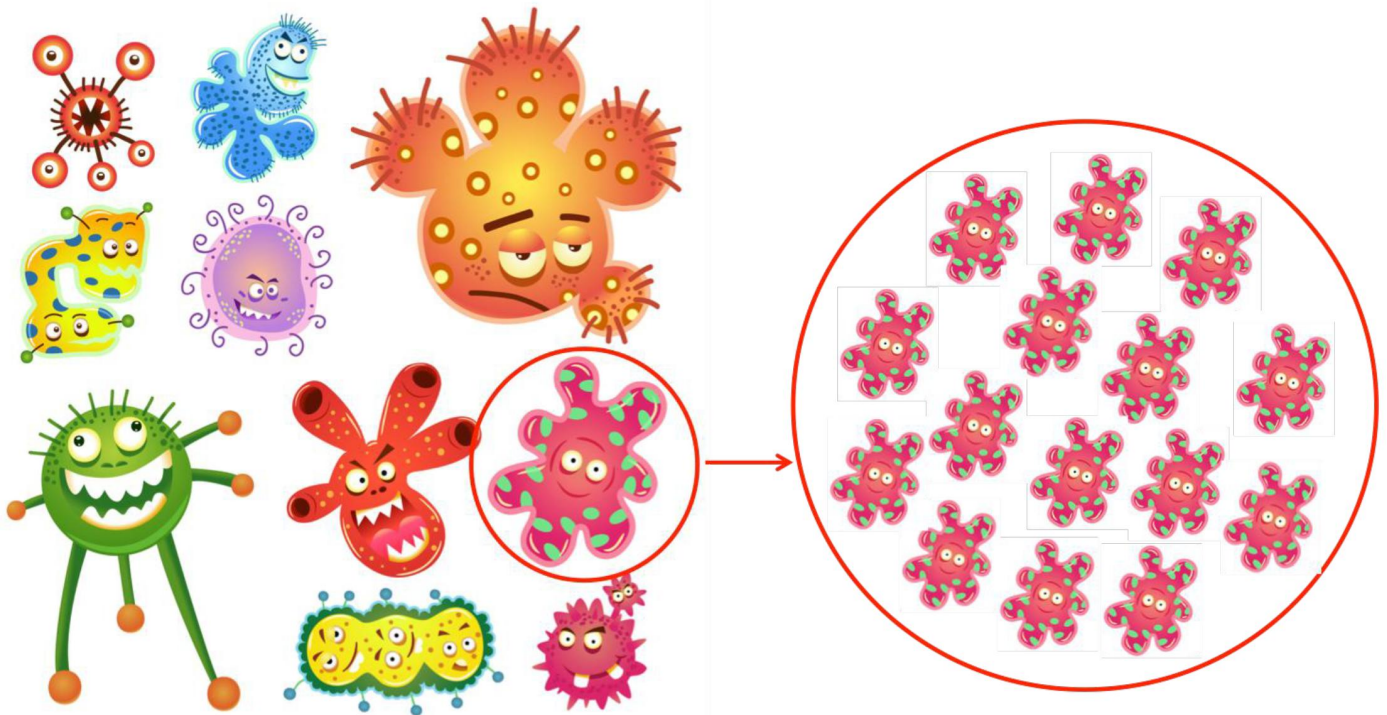
Experiment 7: Leaving food out overnight



We are all guilty of this. Well, at least I am! We leave food on the counter, sometimes overnight, sometimes longer. Is it safe to eat this food? Has bacteria completely contaminated it. Here's how to test this:

1. Take some food and leave it on the counter overnight (or at different time points: 1hrs, 24hrs, 48hrs, etc.). We recommend using “moist” foods like butter, milk, cold cuts, whatever you like.
2. Before doing this, wipe each piece of food with a sterile swab and transfer the contents to an agar plate. This will serve as our baseline control for comparison.
3. After the time is up, transfer bacteria from the “spoiled” food to a separate agar plate using a cotton-tipped swab.
4. Wait for bacteria to grow on the plates using basic instructions on page 4 (steps 6-8).
5. Did leaving food out overnight increase the amount of bacteria? Maybe I should think twice about using butter left on the counter overnight? 😊

Experiment 8: Make a bacteria clone!



It is really easy to clone bacteria! They are single cell organisms that multiply by a process called bacterial fission. Here's how to create a clone!

1. Use a cotton-tipped sterile swab on an object with a lot of bacteria (such as the inside of a toilet). Gently wipe the contents on your agar plate.
2. Grow bacteria using basic instructions on page 4 (steps 6-8).
3. After several days, you should have multiple separate bacterial colonies like the picture below:



4. Use a cotton-tipped swab to pick up a single bacterial colony, and wipe this colony all over a new agar plate.
5. Grow bacteria on this new plate using instructions on page 4 (steps 6-8).
6. After a few days, you should have several bacterial colonies on the new plate. Believe it or not, these are all identical bacteria, because you picked a single colony! It may seem simple, but you have created a bacterial clone!

Experiment 9: Preserving foods



This is an up close picture of salt crystals. Salting is the process of preserving food with dry edible salt and is one of the oldest methods of preserving food. It works because bacteria and other microorganisms cannot survive in a highly salty environment. In this experiment you will test a variety of different substances for their ability to preserve foods.

1. Take several pieces of the same type of food and treat them with salt, spices, or anything else you'd like to test as a preservative. You can try using cold cuts or butter for the food.
2. Make sure to include a control of food not treated with anything for comparison.



2. Leave the treated and untreated food at room temperature for several days at least.
3. Use cotton-tipped swabs to pick up bacteria from the food and transfer the contents onto separate agar plates.
4. Use the basic instructions on page 4 (steps 6-8) to get bacteria to grow.
5. After several days, count bacterial colonies to determine which substance is the best preservative.



Experiment 10: What is the favorite temperature of bacteria?



This is a picture of Grand Prismatic Spring, the largest hot spring in Yellowstone Park. The beautiful colors surrounding the spring are from bacteria and algae, which love the 170° F temperatures. We have found that bacteria grow best on our agar plates between 88 and 100° F. Test this for yourself!

1. Use a cotton-tipped sterile swab on an object with high numbers of bacteria (it's gross, but the inside of a public restroom toilet bowl is a good place to try). Wipe the contents onto an agar plate. Repeat this again for multiple agar plates.
2. Incubate the plates at different temperatures. For example, try 70° F, 80° F, 90° F, 100° F, and 110° F. One way to do this is to place the plates at different distances from a heating source such as a lamp or portable heater. Use a thermometer to check the temperatures.
3. Count the numbers of bacterial colonies growing on the plates after several days.



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