***How Clean is Clean?***

**Understanding the difference between visual and microbial standards of “Clean”**

**Background:**

Many of us see a countertop or food prep surface that is free from dirt or other visual debris and assume that it is clean enough to eat off of. But even *visually* clean surfaces can be harboring pathogenic (disease-causing) bacteria that can end up contaminating our food.

*Hypochlorous acid* (HOCl) is the sanitizing agent present in household bleach. Even though bleach has been used as a sanitizer for decades, the way in which it destroys bacteria has only begun to be understood. In 2008, researchers published a study detailing how HOCl works (Winter J., Ilbert M., Graf P.C.F., Ozcelik D., Jakob U., 2008, Bleach Activates A Redox-Regulated Chaperone by Oxidative Protein Unfolding, *Cell*, 135(4): 691-701). Simply put, HOCl causes certain proteins to unfold and coagulate, rendering them inactive. These proteins are enzymes that are critical for many metabolic functions – without them, the bacteria cannot survive. Because it takes some time for the HOCl to enter the bacterial cells and interact with the proteins, bleach should be allowed to remain in contact with surfaces for a minimum of 2 minutes.

This lesson gives students the opportunity to conduct hands-on microbiology experiments, including environmental swabbing techniques, using prepared media, and counting microbial colonies after incubation; to understand the difference between *clean* and *sanitary* based on microbial counts; and to practice proper sanitizer preparation and application.

Students should be divided into groups of 2-4. Each group will receive 2 swab tubes/bottles and 2 petri dishes. Students will swab an assigned area of the classroom, sanitize the area, and then re-swab their area. After plating and incubating the samples, students will compare the results pre- and post-sanitizing from different areas of the classroom.

**Vocabulary:**

*Clean* – A surface that is free of visible dirt, dust, food waste, or other debris. *Cleaning* is the first step in proper kitchen sanitation.

*Hypochlorous acid* – The active component of bleach, with the chemical formula HOCl (Hydrogen, Oxygen, Chlorine). HOCl works by *oxidizing* several important enzymes in bacteria, causing them to unfold and coagulate (this is similar to the effect heat has on an egg white).

*Pathogenic* – Bacteria that are able to cause disease in humans. Though some forms of microbes may survive the sanitizers commonly used in kitchens, they are not usually able to cause human illness.

*Sanitary/Sanitized* – A surface that is free of pathogenic bacteria. *Sanitizing* is the second step in proper kitchen sanitation.

**This lesson addresses the following USOE Core Standards:**

**Foods & Nutrition Sciences 1**

Standard 1, Objective 4: Identify and apply sanitation rules and guidelines.

**Biology**

Standard 2, Objective 1d: Explain the role of enzymes in cell chemistry.

**Materials**

Prepared petri plates for Total Plate Count (2 per group)

* Check with the biology teacher at your school to see if they have plates and media available
* *Nutrient Agar Plates* (prepared) cost $1-2/plate. They are available through many microbiology and educational sites, such as Carolina.com. If you use agar plates, you will also need plate spreaders.
* *3M Petrifilm*™ Aerobic Count Plates (#6400) cost $1-2/plate. They are available through Carolina.com, or by searching for suppliers on the 3M website (<http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/>). Petrifilm™ plates come with a special spreader and specific instructions for their use.

Swabbing kits (2 per group)

* Environmental swabbing kits (4-10mL) are preferable, but medical swab kits (1mL) will also work.
* 3M offers several environmental swabs, which average $1/swab. Two recommended swabs are the *Swab-Sampler with Buffered Peptone Water* (#RS96010BPW) and the *Quick-Swab* (#6432). They are available by searching for suppliers on the 3M website at the above link, and should be used according to the provided instructions.
* BD offers many environmental and medical swabs ([www.bd.com](http://www.bd.com)). Currently, only the *CultureSwab Plus* is available through Carolina.com, but others may be available through other educational websites.

Unscented household bleach

Buckets or containers to mix sanitizer (1 per group)

Bleach test strips (1 per group)

* Test strips are available through restaurant supply and specialty kitchen stores. They can also be found through many websites. They typically cost $5-7/tube of 100.

**Instructions** *(with teacher notes)***:**

1. Swab your assigned area following your teacher’s instructions. Return the swab to the bottle or tube. Make sure to label it “pre-sanitized.”
   * *Suggested areas include food prep counters, stovetops, cutting boards, light switches, desks, and worktables.*
   * *Depending on the type of swab you are using, demonstrate for the students how it should be used (follow manufacturer instructions). You can swab your desk and plate it to show them how clean your desk is!*
   * *For students swabbing large surfaces such as a countertop, you can have them swab a 4-5” square area. A square can be made with electrical tape on the countertop before the pre-sanitized swab is taken. Have the students be careful not to pull the tape up as they sanitize the area, then the same square can be used again for the sanitized swab (Step 5).*
2. Mix bleach water as instructed by your teacher. Test the bleach water with a test strip. Compare the color of the strip to the chart on the test strip container. The bleach should be between 50 and 100 parts per million (ppm).

* *To mix bleach at approximately 100pm, use 1 tsp of unscented household bleach per quart of cool water. This is the maximum residual level allowed by the FDA Food Code. Point out that 100ppm is based on the hypochlorite concentration, not the amount of bleach that is added. Fresh household bleach is typically 5% hypochlorite. Old bleach can still be used, but more than 1 tsp will need to be added.*
* *Hot water should not be used for sanitizers. Hot water causes the bleach to break down quickly, so it loses its sanitizing ability. The best temperature for bleach sanitizing solution is 70-80°F.*

1. Put a cleaning rag or sponge into the bleach solution. Let it sit for 1-2 minutes, then wring out the extra solution so the rag is very damp but not dripping. Clean your assigned area thoroughly with the rag or sponge. Let the area air dry for 2 minutes.
2. Rinse your rag or sponge in warm running water. Wring it so it is just damp, then wipe off your sanitized area to remove any extra bleach.
3. Using your second swab kit, swab the sanitized area the same way you took the swab for Step 1. Return the swab to the bottle or tube. Make sure to label it “sanitized.”
4. Tighten the lids on the swab tubes and shake them as instructed by your teacher.
   * *Show the students how long and vigorously the tubes should be shaken based on manufacturer instructions.*
5. Transfer the liquid from your tubes to labeled petri dishes as instructed by your teacher.
   * *Show the students how to transfer liquid to the petri dish and spread it, using your example swab. Make sure to follow the manufacturer recommendations for the plates you are using.*
6. Give your labeled petri dishes to your teacher. They need to be incubated for about 2 days.
   * *If you have access to an incubator at your school, this is the best place to leave the plates. An incubation temperature of 30°C (85°F) is recommended.*
   * *If you do not have access to an incubator, stack the plates in a storage container with holes punched in the lid. Put the storage container in a warm area in your classroom for 48 hours. If you have a washer and dryer in an enclosed room and will be washing laundry so the room stays warm and humid, this would be a great spot to leave the container.*
7. Get your petri dishes back from your teacher. Count the number of colonies that have grown on your plates. Using a black marker or gel pen, put a dot over each colony as you count it. If you have a plate with too many colonies to easily count, ask your teacher how to calculate the number of colonies that have grown.
   * *If you have petri dishes with numerous (>100) colonies, have the students draw a 1mm square on the lid. They can then count the colonies only inside the square, and multiply the number by the area of the plate (this will vary depending on the plate you are using, but the manufacturer should list the exact size of the plate on the box or in the user guide).*
8. Compare the number of colonies in the pre-sanitized and sanitized plates. How dirty was your area at first? How effective was the sanitizing procedure? Compare your results to the rest of the class. What were the dirtiest areas of the classroom?
   * *Make a chart of each of the areas with “pre-sanitized” and “sanitized” columns. Have the student groups fill in the data as they finish counting colonies.*
   * *Stress the importance of proper hand washing before doing any food prep. Often, the dirtiest part of a classroom are the light switches and door knobs/handles – these are touched by many people, don’t always get cleaned regularly, but we don’t usually think of them as being dirty!*

**Discussion points/questions:**

1. How does bleach work? Why can’t bacteria survive without enzymes?

* *Hypochlorous acid is the active ingredient in bleach. It reacts with proteins in the bacteria and causes them to change shape then group tightly together (coagulate). This is similar to what happens when an egg white is cooked.*
* *Enzymes are special proteins that are used by all living things. Enzymes are needed for all of a cells functions, including digesting food sources, repairing damage, and building new proteins and enzymes. Enzymes need to be in a certain shape to be useful. If this shape changes, the enzyme might not work as well or it might not work at all. Just like it is impossible to change a cooked egg white back to a raw egg white, if enzymes begin to coagulate, or group together, it is impossible to change them back to the way they were before. When this happens quickly or when many different enzymes are affected, the bacteria cannot create enough new enzymes to survive.*

1. Were there some surfaces that were dirtier than others? Were there some surfaces that were harder to sanitize than others? How effective is bleach?
   * *Sometimes the dirtiest surfaces are the ones we don’t even realize we are touching. This shows how important it is to practice proper hand washing and kitchen sanitation.*
   * *Very solid surfaces (like stainless steel) are easy to sanitize because the bacteria stay on the surface. This allows the bleach to be in contact with the bacteria for a longer amount of time, so they are more likely to be destroyed. Porous or cracked surfaces (like wood) are more difficult to sanitize because bacteria can work their way deeper into the pores and cracks. It is possible that the bleach will not reach all of the bacteria that may be hiding in the pores. It might seem that this would also keep the bacteria from reaching food that is being prepared, but when moisture is present the bacteria are more likely to work their way back to the surface. This means they may survive in these porous materials, then find their way back into our food during preparation. This is why wood cutting boards and utensils are not recommended in commercial kitchens.*
   * *Bleach is one of the most effective sanitizers available, but it is not perfect. Some kinds of bacteria may still survive, especially those than can form spores. The test we performed will not show us how many spores were present on the surfaces we tested, though.*

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To sanitize surfaces, they first need to be cleaned. Then they need to be sanitized. Bleach is one of the most effective, inexpensive sanitizers available. But even though bleach has been used as a sanitizer for decades, we only recently understood how it worked. We now know that *hypochlorous acid*, the active ingredient in bleach, attacks *enzymes* in bacteria. Enzymes are special proteins that are necessary for all livings things. Without active enzymes, bacteria cannot survive.

**Instructions:**

1. Swab your assigned area following your teacher’s instructions. Return the swab to the bottle or tube. Make sure to label it “pre-sanitized.”
2. Mix bleach solution as instructed by your teacher. Test the bleach water with a test strip. Compare the color of the strip to the chart on the test strip container. The bleach should be between 50 and 100 parts per million (ppm).
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10. Compare the number of colonies in the pre-sanitized and sanitized plates. How dirty was your area at first? How effective was the sanitizing procedure? Compare your results to the rest of the class. What were the dirtiest areas of the classroom?

**Questions for Discussion:**

1. How does bleach work? Why can’t bacteria survive without enzymes?
2. Were there some surfaces that were dirtier than others? Were there some surfaces that were harder to sanitize than others? How effective is bleach?