Grace:

**November 21, 2020**

Audrey and I - Video conference meeting with Dr. Beatriz Garcia-Diaz

Purpose: to decide how to set up the experiment and culture bacteria.

1. **How to do the cultures?**

Two ideas discussed:

1. Measuring zones of inhibition on bacterial “lawns” on agar plates

-make a “lawn” of bacteria on the agar plates by growing bacteria until the plate is completely covered

-next put discs dipped in test solutions onto the lawns. Wait until the bacteria around these discs dies. Then measure the clear rings (bacteria-free) around the discs = zone of inhibition.

-then compare the sizes of the rings. The bigger the ring, the better the solution was at killing the bacteria.

-the problem with this technique = not possible to test different concentrations of the test solutions.

1. Modified-use dilution method with liquid (broth) cultures (see video from Dr. Amer Al-Jawabreh)

-he describes taking liquid cultures that contain E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis (grown in tryptic soy liquid broth for 24-48 hours) and putting a small amount of liquid on the glass slides. Once the bacteria on the slides had dried, he dipped each slide into 25ml of disinfectant solution in a beaker and left it for 1 minute. He then let the slides air dry once more. Last, he put each slide in 20 ml of tryptic soy broth in 25-50ml tubes and incubated them for 24 hours before he tested the turbidity.

-one alternative method would be to put the disinfectants directly on the tiles without dipping as dipping might wash the bacteria you need off. You could spray, wipe, or lay a cloth soaked in the disinfectant on top of the tiles for a set amount of time.

-put each tile in a tube of liquid culture broth and let the remaining bacteria on the tile grow inside the broth. Might have to agitate the broth to be sure enough oxygen gets in the tubes for aerobic kinds of bacteria to grow.

-after enough time for bacteria to grow, use a spectrophotometer to measure the turbidity (or cloudiness) of each tube (borrow equipment from Dr. Garcia-Diaz). Will get a number reading that corresponds to the amount of bacteria growing in the liquid broth. This way, you can easily measure the number of bacteria without counting colonies.

**2. What source of bacteria to use?**

-use a swab to collect bacteria from the bottoms of shoes, school or home showers, hands, food.

-from past experience (Science Camp, other students’ Science Fair projects), the most bacteria can be found in shower stalls and the bottom of shoes.

**3. What materials are needed?**

-Dr. Garcia-Diaz may have some materials we can use in the school science lab (glass slides and tubes, incubator)

1. Culture medium?

-nutrient agar plates and broth?

-tryptic soy agar plates and broth?

-both kinds should allow many varied types of bacteria to grow (see reference)

-most common bacteria tend to be aerobic (need oxygen to grow) so they might only grow in the very top layer of a liquid (such as the broth). To help the bacteria grow, we may need to either agitate the tubes (by hand or using the lab’s agitator) to be sure oxygen gets into the liquid or use containers with large areas to make a bigger surface with exposure to oxygen.

-Dr. Garcia-Diaz noted that E. coli will grow in the liquid broth without agitation

-advantage of using liquid broth to make an initial bacterial culture to use for all tests = it creates a regulated solution that can then be used on all tiles or plates being used so that the same bacterial mix is used for all trials.

-should do initial testing to be sure that the test bacteria grows on the agar plates and in the liquid broth.

1. Tubes and tiles/slides?

-need to check with lab supply company (Dalynn Biologicals) to see what is available

-could use metal discs from school lab, tiles or glass slides

-need to make sure that the slides/tiles fit in the test tubes if we are going to use the modified-use dilution method

1. Swabs?

-need sterile swabs to put in sterile saline to collect initial samples. Dr. Garcia-Diaz has some swabs in the school lab.

-check with Dalynn Biologicals about our options, prices, and how soon we can collect the materials

**4. Phases of experiment**

-will most likely need about 48 hours for each step of bacterial culture to give the bacteria enough time to grow

1. Initial trial

-will test different sources of bacteria and different culture plates/broths

-swab different areas to see how much bacteria can culture

-try growing the bacteria on the agar plates and liquid broth to make sure that it will grow

-consider taking a culture plate and broth home to see how well the bacteria will grow under home conditions. Could try at room temperature (20-21 degrees Celsius) or make a home incubator (using a lightbulb and an insulated box).

1. Main experiment

-use single full-strength concentrations of test solutions

-use a standard exposure time to the test solutions

-need around 3-4 trials (tiles/slides) for every test solution plus control group

1. Possible second phase

-if you cannot find a difference between the test solutions, think about repeating the experiment (3-4 trials for each set-up) with a different concentration of the test chemicals or a different time of exposure to the chemicals

**5. Areas to research**

1. What is meant by “disinfectant”? How do the different chemicals work to kill bacteria?
2. What is the difference between killing bacteria and killing viruses? Would the same chemicals be useful for both?
3. What are possible harmful effects of the cleaning solutions used?
4. What are the other real-life considerations - environment and economic? For example, what is the cost of each type of disinfectant? If you need a lot more of a natural disinfectant to have the same result, is the cost worth it? Which disinfectant are we recommending?
5. What are the main active ingredients in natural and artificial disinfectants?
6. How can disinfectant activity be measured in a lab?

**6. Other thoughts:**

-have to be prepared to do the experiment at home if we end up in quarantine or schools are temporarily closed due to COVID

-can culture bacteria at home. Could use an incubator if needed using materials at home.

-could also do some parts of the experiment at school and some at home. Could possibly culture the bacteria at school using the incubator at school but bring the samples home to do the counts (can borrow the spectrophotometer).

-if doing the experiment in the school lab, need to arrange meeting times with Dr. Garcia-Diaz - in the mornings (7:30-8:00), after school (except Tuesdays), or at lunch

-keep bibliography of articles used for research

-try to start doing initial testing for information on the experiment setup in the next couple weeks. Decide on the bacterial sources, method to use, type of culture media, buy and collect materials.

**November 23, 2020**

Ideas for initial testing phase to find out where can get samples that give enough bacteria and whether culture materials work or not:

Materials needed:

-hand sanitizer

-ethanol (for cleaning equipment)

-sterile swabs

-sterile saline to moisten swabs before use

-agar plates - tryptic soy (approximately 10)

-tryptic soy broth - 250ml bottle

-50 ml sterile Falcon tubes (for doing liquid cultures, approximately 10)

-racks to support the tubes upright

-timer

-spectrophotometer

-incubators

-school lab incubator with agitator (may improve oxygen in broth and help bacteria growth)

-home - make from styrofoam cooler, thermometer, light or low-heat warming pad

-gloves

Procedure for initial testing:

-make sure hands are clean (hand sanitizer between steps or clean gloves)

-prepare the tubes with 10 ml of tryptic soy broth trying to maintain sterile conditions inside the tubes (clean hands before).

-label agar plates and liquid broth tubes with locations for each sample to be taken

-collect bacteria from 3-4 locations:

-take a sterile swab (being careful not to touch the tip). Moisten it with sterile saline.

-roll swab around test area to coat well with possible bacteria (?15 seconds)

-apply some swabs immediately to agar plates - use back and forth zig-zag technique to cover whole plate. Could turn once by 90 degrees to do second zig zag.

-put other swabs into the liquid broth culture tubes

-do 2 plates for each location - 1 will be incubated at school and 1 at home

-cover agar plates with their lids, put liquid broth tubes in tube racks so they stay upright, and put the plates and tubes in the incubators.

-set the school incubator to agitate the tubes continuously.

-4 times per day (6:30am, 12:30pm, 6:30pm, and 12:30am) swirl the broth in the tubes in the home incubator vigorously (?60 seconds each) to help add oxygen. Ask for help from our parents for 12:30pm and 12:30am agitations.

-allow the bacteria to grow for 48 hours

-then examine each tube and agar plate individually. Consider photographing them or writing descriptions. Use spectrophotometer to measure the turbidity after agitating the tubes to distribute the bacterial colonies in the liquid.

Possible locations to sample:

-shower stall at home or school

-school locker room

-kitchen counter at home (where exactly?)

-hands

-bottom of our shoes

-phone

Once we complete this initial testing, we can then decide on our final experiment set-up.

-need to build and test a home-made incubator in the next 1-2 days. See [www.sciencenewsforstudents.org/blog/eureka-lab/five-second-rule-growing-germs-science](http://www.sciencenewsforstudents.org/blog/eureka-lab/five-second-rule-gorwing-germs-science) for an idea.

**November 24, 2020**

We decided to do some initial trials to find out if it was possible for us to grow bacteria on agar plates and in liquid broth because we do not know if we will have to isolate at home unexpectedly or not. We are planning to try some initial tests in the school lab and at home. We would rather do our experiment using the school incubator but we have to have a backup plan if we cannot go to the school because of the pandemic. Bought supplies to make home incubator (styrofoam cooler, 40W lightbulbs, light socket with plug, and thermometer with temperature probe on a wire) Put together homemade incubator by taping the probe of the thermometer so it ended midway down one of the bigger sides of the box. On the opposite side of the cooler, taped the light on an extension cord to the middle of this side. Put cooler in basement laundry room and left it overnight to see how warm it got in the styrofoam box. To adjust the temperature to stay between 30-35°C (to match school lab incubator) I opened or closed a gap in the lid to let some of the heat out. If I kept a 6 cm gap along the longer side of the cooler, the temperature stayed around 33°C.

**November 25, 2020**

Audrey’s mom went to Dalynn Biologicals, a company that sells equipment to do experiments involving microbiology, to buy agar plates and liquid broth for our initial trials. The microbiologists at Dalynn recommended that we use tryptic soy agar and broth. We also got some test tubes, sterile swabs, saline, glass slides, and a spectrophotometer from Dr. Garcia-Diaz. Audrey is going to do a test trial with the agar plates and liquid broth we got to see if she can culture bacteria from the bottoms of her school shoes using the school lab incubator. I am going to do a similar trial at home using my homemade incubator. We were just told that we will have to go to online learning starting on November 30.

**November 29, 2020**

I read the procedure that Audrey had written up on her testing in the school lab and spoke to Dr. Garcia-Diaz for extra tips. I then wrote a procedure for my home trial. Audrey discovered that she got some bacterial growth for her shoe swabs on both the agar plates and in the broth in the school lab. There is an agitator in the school incubator that continually moves the broth slightly back and forth to help add oxygen to the broth (to help aerobic bacteria grow) and mix the bacteria around. Even though I can keep the temperature in my incubator in a similar range to the school incubator, I do not have a way to constantly agitate the broth at home. I will have to see if I can grow bacteria just as well at home. I will try to agitate it by swirling the broth around every 4 hours by hand and compare the results to how well bacteria will grow in broth that is not agitated. I let my incubator warm up for several hours, gathered my supplies and equipment and started my first trial. I swabbed my school dress shoes and gym shoes that I brought home. Audrey and I are talking about our procedure and taking notes, which we will write in another entry. I am planning to do my cultures for 48 hours then look at what I could grow in that time visually. I will also use the spectrophotometer on the liquid broth to practice getting readings from the broth.

Started an initial test in my home incubator:

-made 3 agar plates: 1 control, 1 from swab of black school shoes, 1 from swab of gym shoes.

-made 3 broth tubes: 1 control, 2 from swabs of black school shoes (1 will be agitated approximately every 4 hours, 1 will be kept still). To prepare the tubes, I removed broth from fridge, opened the bottle and flamed the top briefly with lighter. I then poured 5 mL of liquid into each tube directly (being very careful not to touch the top of the tubes with the broth bottle).

-when swabbing for the control agar and test tube, I dipped the swab in saline solution but did not swab anything, I only applyed the swabs to the culture agar or broth. Used a new saline ampule for each agar or tube set-up.

-to get the cultures from the shoes, I wet the tips of the swabs with saline (a new ampule for each swab) and swabbed the bottom of the shoes for around 30-60 seconds before rubbing the swabs on the agar plates (zig-zagged 4 times across each plate) or in the broth (put swab into tube, cut off stick with sterilized scissors and left swab in broth).

Observations:

Nov. 29:

-2pm - agar plates and culture tubes placed in home incubator. Temperature at 31-34 degrees Celsius (checked often and adjusted by opening or closing gap at lid).

-6pm (4 hours) - no visual change, swirled broth in “agitated” tube for about 1 minute. Temperature 33 degrees.

-10 pm (8 hours) - no visual change, swirled broth in one tube to agitate. Temperature 32.7 degrees.

Nov. 30:

-1 am (11 hours) - no visual change in any plates or tubes, swirled broth in one tube to agitate. Temperature 32.8 degrees.

-6 am (17 hours) - a few colonies starting on both shoe agars. Broth from shoe swabs both starting to get cloudy - agitated tube a bit more cloudy than still tube. Temperature 32.5 degrees.

-10 am (21 hours) - swirled broth in one tube (forgot to record how cloudy or the temperature).

-2 pm (24 hours) - swirled broth in one tube (forgot to record how cloudy or the temperature). Could not analyze then as busy with school work.

-6pm (28 hours) - swirled broth in one tube but again did not make any observations.

-10 pm (32 hours) - analyzed all cultures and ended this test. The temperature in the incubator was 32.8 degrees.

Results at 32 hours:

-agar plates - no visible colonies on control swab plate. Both shoe plates (black school shoes and gym shoes) had small colonies (perhaps 50-100 each). There was no significant difference in the number of colonies from the different shoes - but the black school shoes grew mainly white coloured colonies and the gym shoes grew more orange colonies.

-broth culture tubes - all 3 tubes (including the control) were equally cloudy in appearance. The agitated broth tube from the shoe swab had a solid white substance on the top of the broth. It broke up into chunks when agitated but it was not possible to completely break it down in the broth.

Spectrophotometer readings of broth samples from tubes (absorbance at 600 nm at 32 hours growth):

-Control: 0.800

-Shoe swab - still: 0.543

-Shoe swab - agitated: 0.540 (but had big chunks floating in the broth that would not have been picked up by the analyzer)

Conclusions:

-the control swab on the agar did not grow any bacteria but the control swab in broth did (and had a higher absorbance suggesting more bacteria) than the 2 shoe swabs. I used the same technique to produce both cultures except used different swabs (from the same package) and different saline ampules to moisten the tips of the swabs. I did notice that my saline ampules were past their expiry date and some had dried up. I wonder if the saline ampule I used to make the control broth tube could have been contaminated. Other ways in which the control broth could have been contaminated include: the tube not being completely sterile inside or I contaminated the culture accidentally when I prepared it (although I tried to be very careful). I will plan to get brand new sterile swabs and saline tubes for my real trials and be extra cautious with my technique.

-I also learned I do not have to agitate the tubes in my incubator as the tube that was not agitated grew almost as much as the tube I did not.

-32 hours is too long for the cultures as the one tube grew a solid portion that was not possible to analyze accurately with the spectrophotometer. I should look at shorter culture times.

**December 20-21, 2020**

Started another run to gather more information on how to best set up our final experiment.

Part 1:

-1 am (Dec. 20) - did new swabs to produce new broth cultures (did not use any agar plates). Techniques:

-used same saline ampule to moisten the end of all swabs (in the previous

trial November 29th, I used a different ampule for each plate or tube).

-instead of leaving the end of the swab in the broth tubes for the whole culture, I

swirled the tip of the swab in the broth in the tube (5mL) for 30 seconds before removing and throwing away the swab. So the broth cultures were run without the swabs remaining in the tubes.

-I used the same home incubator set-up aiming to keep a temperature of around 33 degrees.

-made 1 control tube (swab tip dipped in saline only)

-4 other tubes - each made with different swabs. For each tube, a new swab was dipped in the saline (the same saline ampule was used for all 5 tubes including the control). Then each swab was used to smear around the edges of my kitchen sink just under the granite lip to collect bacteria. The tubes were labelled: “12 hours”, “16 hours”, “20 hours”, “20 hours master broth”.

-the tubes were placed in tube racks in the home incubator with the temperature kept between 32-34 degrees. None of the tubes were agitated.

-I analyzed the turbidity at 12, 16, and 20 hours using the spectrophotometer. To do so, I poured the contents of the tube labelled to be sampled at the designated time into the test cartridges. Once the analysis was done for that tube, it was then discarded. I only analyzed the control tube after 20 hours of culture. The tube labelled “20 hours, master broth” was not analyzed for an absorbance reading but was then used to transfer to slides in the second part of this test.

-my results for turbidity:

-12 hours: 0.528

-16 hours: 0.620

-20 hours:

-control tube: 0.015

-sink swab tube: 0.830

-the second remaining sink swab tube was kept to use for creating slides for the second phase and the turbidity was not analyzed. It appeared about equally as cloudy.

Part 2 (December 21):

Technique used:

-hand hygiene with initial thorough washing then regular use of alcohol-based hand sanitizer between each step

-set up 6 culture tubes each with 10 ml fresh tryptic soy broth (flamed top of broth bottle between each tube and before closed). Tubes placed in tube rack.

-labelled tubes:

-control 1, control 2

-Method 1, Method 2

-bleach 1, bleach 2

-set-up clean work surface (tin tray sprayed with isopropyl alcohol to disinfect). Arranged 6 slides (wiped with isopropyl alcohol on paper towel) on this clean work surface - in groups of 2. One slide will go in each culture tube.

-created a paper template to match the size of the slide with 1” marked off. Placed next to the clean work surface but not directly under the slides. Will use to estimate the 1” area at the end of the slides where the bacterial culture liquid will have to be placed.

-got final remaining broth culture from first phase of experiment (broth at 20 hours)

-prepared 2 125mL sterile jars with the disinfectant solutions to be tested. One jar had Method Antibac Disinfecting All-Purpose Cleaner spray (5% citric acid). The other jar had Fantastik Disinfectant All-Purpose Cleaner With Bleach (3% sodium hypochlorite). Both sprays were purchased within the last week.

-using the same broth culture (20 hour culture), the same sterile 10uL plastic loop was used to transfer a small amount of the culture liquid to the lower 1” of each slide on the clean work surface (using the template nearby to estimate the application area). The liquid was spread evenly over the square area coming close but not over the edge of the slides. The slides were left undisturbed until the liquid had dried completely on each (about 10 minutes).

-once the bacterial cultures had dried, the 2 slides for the control (untreated) group were placed in their labelled culture tubes with the 1” end with the culture resting into the broth).

-1” squares of coffee filter material were prepared (one for each of the 4 slides to be exposed to the disinfectants).

-using a sterilized metal forceps (flamed briefly), one filter square was picked up and dipped into the disinfectant solution to be applied so that the square was completely soaked. Then the square was lifted up and the excess fluid allowed to drip off. It was then applied to the 1” segment of the slide with the bacteria. The disinfectant-soaked paper was gently tapped down to rest completely on the surface of the slide and left in place for 2 minutes before being removed and discarded.

-this same procedure was used for the remaining 3 slides (with 2 slides being treated with the Method cleaner and 2 with the Fantastik cleaner). The forceps were rinsed with water and flamed before each new slide.

-the residual liquid cleaner left behind on the slides when the papers were removed was allowed to dry completely (took about 15 minutes). Then each slide was transferred to the correct labelled culture tube using the sterilized forceps.

-the caps were placed loosely on the tops of all 6 tubes and the tube holder with these tubes was placed in the home incubator.

-the temperature in the incubator was kept between 31-34 degrees and the tubes left undisturbed for 16 hours. At 16 hours, the liquid in each tube was agitated to evenly distribute any material inside and then the turbidity analyzed with the spectrophotometer.

Results (16 hour cultures):

1. Control (untreated): mean 0.593
   1. Tube 1: 0.525
   2. Tube 2: 0.478
2. Method (citric acid) treated: mean 0.427
   1. Tube 1: 0.376
   2. Tube 2: 0.478
3. Fantastik (bleach) treated: mean 0.357
   1. Tube 1: 0.330
   2. Tube 2: 0.384

What I learned from this test:

-the turbidity continues to climb from 12 to 20 hours. I decided to use a 16-hour culture time for my second phase as I did get some solid clumps in my November 29th test so I was worried about having too much bacterial growth if I waited too long.

-there was again some growth in the control (saline-only) tube in the first phase although it was not as much as in the November 29th test. I was using the old saline ampules and swabs again and wonder if they are not completely sterile. I plan to use new sterile supplies for our final experiment.

-I got a similar turbidity reading in the untreated (control) tubes at 16 hours (mean of 0.593) as in my initial phase culture (0.620) suggesting that the bacteria are still alive and will grow if transferred from the initial broth to the second phase culture.

-it takes a long time for any liquid applied to the slides to dry (at least 10-15 minutes)

-I did get quite a bit of bacterial growth in all 4 treated slides but there was less than in the control group. The bleach did seem to perform better than the Method spray as there was a lower mean absorbance in the bleach tubes.

-I noticed that the 10 mL of broth only covered about the lower ½” of the slides. So in the future, I should only treat the lower ½” (instead of 1”) of the slides - or use more broth in the tubes. I don’t think it would have made that much of a difference in my results though as I did treat the whole 1” with disinfectant.

-I am a bit surprised I still got quite a bit of growth with both disinfectants. I did see a difference between groups. I wonder if I should use bigger pieces of filter paper to make sure I cover the whole area where the bacteria were applied. I could also try to keep the bacterial culture liquid to a smaller area in the centre of the lower ends of the slides. Additionally, I can consider leaving the disinfectant-dipped papers on top of the slides for longer than 2 minutes to get more of an effect. However, the technique I used probably does mimic real life (except I did not wipe the surface of the slides) as people don’t soak the surface they want to clean for a long period of time (even though the instructions on the bottles of cleaner do say to leave it wet on the surface to be cleaned for 10 minutes for full effect).

-I should also think more about how to make sure my slides and work surfaces are sterilized before the experiment to reduce contamination.

**January 4, 2021**

Looked at all the liquid all-purpose cleaning products at Safeway and Blush Organics that were labelled as a “disinfectant” or that “killed 99%+ of germs/bacteria” to get an idea of the active ingredients in things that can be easily purchased at stores. Here is a list of active ingredients in these products:

1. Glycolic acid 1.75% (Original Pine Sol)
2. Thymol 0.05% - from thyme oil (Seventh Generation Disinfecting Multi-Surface Cleaner)
3. Citric acid 2.5 or 5% (in several products - Lysol Bathroom Foam Cleaner, Compliments Disinfectant Bathroom Cleaner, Method Antibac Disinfection All-Purpose Cleaner, EcoMax Tea Tree Disinfecting Toilet Bowl Cleaner)
4. Quaternary ammonium chlorides (di/n/alkyl dimethyl benzyl ammonium chloride, didecyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride, octyl decyl dimethyl ammonium chloride) (in a large number of products including one labelled as “natural”, in varying concentrations - Spray Nine Heavy Duty Cleaner/Disinfectant, Attitude Nature Technology Disinfectant Cleaner, Lysol All-Purpose Lemon Cleaner/Disinfectant, Lysol Power & Fresh Multi-Surface Cleaner, Microban 24 Hour Multi-purpose Cleaner/Disinfectant, Compliments Antibacterial Multi-Surface Cleaner, Compliments Multi-Surface Disinfectant, Fantastik Original All-Purpose Cleaner/Disinfectant)
5. Sodium hydroxide (lye = alkali) 0.34% (Mr. Clean Disinfectant)
6. Sodium hypochlorite (bleach) 2-3% (in several products, labelled as “bleach” - Lysol Bathroom Bleach Cleaner, Fantastik All-Purpose Cleaner/Disinfectant with Bleach)
7. Hydrogen peroxide 0.5% (Natura Solutions Soft/Hard Surface Sanitizer/Disinfectant). It was also in a disinfectant that contained multiple active ingredients (Natura Solutions All-in-One - 3% hydrogen peroxide with potassium persulfate 0.005%, silver 0.001%, lipase 0.35%, protease 6.3%, amylase 0.35%)

I did not find any products containing pine oil although it has been used as a disinfectant in cleaning products in the past. Ethanol or isopropyl alcohol-containing wipes and sprays are also available although are not in the cleaning aisle at the grocery stores. I know that these wipes are used in hospitals for cleaning surfaces. Acetic acid (vinegar) is available in spray bottles in the cleaning aisle as well.

I also talked to Audrey and to Dr. Garcia-Diaz by FaceTime to discuss some parts of our final procedure. We decided to do a few other small tests:

1. How much broth is needed to cover ½” and 1” of the end of a glass slide immersed in the culture tubes? We need to make sure that we cover the whole part of the slide where we applied the bacteria with the culture broth. I noticed that 10mL of broth will cover a little bit higher than ½” (or 1.25 cm) of the end of the slide. I would need at least 15mL of broth to cover 1” (or 2.5cm) of the bottom edge of the slide. Dr. Garcia-Diaz recommended using a maximum of 12 mL of broth when attempting home cultures where we cannot continuously agitate the broth to help oxygenation. Audrey and I decided to use only the bottom 1.25cm of the slide for our area where we apply the bacteria and then dip this end of the slides into 10mL of culture broth in the tubes. We want to avoid having the bacteria inoculum come right to the edges of the slide. So we will apply the bacteria in an area that measures 1.1x2cm. We will make a template to put under the slide as we spread our inoculum to make sure we keep inside this area.
2. What size of loop to use for applying the bacterial liquid inoculum to the slides? We tested 1uL and 10 uL loops (the sizes available for purchase). The 10uL loop (that I have used for my initial testing) makes quite a large puddle of liquid on the end of the slide which takes 5-10 minutes to completely dry. The 1uL loop will apply enough liquid to cover the area we want on our slide. This amount of liquid dries in less than 3 minutes.

Audrey and I also decided the following things:

1. Will put the disinfectants on our slides using the filter paper technique instead of dipping the end of the slide into a container of disinfectant. This is because we believe the filter paper technique is more similar to cleaning techniques used in real life and we are concerned that dipping the slides into liquid disinfectants might wash off some of the inoculum rather than killing the bacteria. Although the instructions on most disinfectants say to apply the disinfectant to the desired surface to be cleaned for 10 minutes, we think that it would be unusual for consumers using these products at home to let the disinfectants sit on a surface for this long. Therefore, we will apply the filter papers soaked in disinfectants to the slides for 5 minutes.
2. We decided to let the pieces of filter paper sit in our test disinfectants for 1 minute before putting them on the slides to try to kill any bacteria that might already be on the filter paper (as the filter paper will not be sterile).
3. We decided to cut the filter paper pieces that will be applied to the slides so that they cover the whole end of the slide with extra coverage to make sure all the bacteria inoculum is treated with the disinfectant. Therefore, in order to cover an inoculum area of 1.1x2cm, we need to cut the filter paper so that it measures 4x2.5cm (the slide is 2.5 cm wide as well).
4. Should we do one single experiment run using 3 slides for each test situation (for a total of 3 trials)? Or 3 experiment runs using 1 slide per run for each test situation (for a total of 3 trials)? We decided to go with the first option as it would keep the conditions controlled for all 3 slides in every group. We could also get the results more quickly so we could have enough time to do more runs if we decide we need more trials.
5. If we can go back to in-person classes in another week, should we do our experiment in the school lab? Since we already have a process that seems to work using our home-made incubators, we will do the experiment at home although we could use the lab. It may also be difficult to schedule the important parts of the experiment during school hours as each culture can only run for a very short time period. It is much easier to time the experiment correctly at home over a weekend.
6. How to split up the experiment so that we both do our part? Audrey will test 3 disinfectants and I will test the 3 left. We will both use the same master broth for our innoculum. We will both use the same controls (slides with no inoculum and no disinfectant treatment, slides with inoculum but no disinfectant treatment). If we find a large, critical difference in our standardized controls, we can then do the experiment again but switch the disinfectants we use so Audrey tests the ones I originally used and I test hers.

**January 8-10, 2021:**

Audrey and I did a run of the experiment. Each of us tested the 3 different disinfectants. We both made initial cultures by swabbing our kitchen sinks and incubating 2 5 mL tubes of these swabs each. Then we combined all 4 tubes together, mixed well, and gave each of us a tube of 10mL of this master culture for the second step of the experiment.

January 8th:

-10:19pm - placed my 2 tubes of 5mL broth produced from swabs of my kitchen sink and 1 control tube (saline-dipped swab only) into the incubator

-I also tested a micropipette device provided by Dr. Garcia-Diaz to see if it dispensed consistent amounts of liquid. I wanted to know if it would be better to use for getting a standardized amount of inoculum on my slides compared to using the 1 or 10 uL loops. This micropipette device can be set to provide a desired amount ranging from 1-10uL. I tested using this device to apply 2 and 5 uL of milk to several glass slides. It seemed like 5uL provided the best coverage to the 1x2cm area on my slide (2uL seemed too little). It took around 4-5 minutes for this amount of milk to dry on the slide.

January 9th:

-overnight, I checked the temperature in my incubator and it was always in the range of 32.8 - 33.7 degrees Celsius.

-at 2:15pm, I removed my 3 tubes from the incubator. The control broth was clear and the other tubes cloudy with a few particles. My mother took the 2 tubes inoculated with the kitchen sink swabs and met with Audrey’s father. They combined all 4 tubes into one master broth then divided the liquid into 2 tubes. I got one tube and Audrey took the other.

-at 3:22pm, I used the spectrophotometer to test this combined master culture and my original control tube. I got the following results:

Master culture (combination of cultures from Audrey’s and my sinks): 0.578

Control broth (saline dipped swab): 0.001

-I then proceeded with the second stage of the experiment, making the slides and placing them in culture tubes.

-I dipped my glass slides in 99% isopropyl alcohol then set them upright in a Pyrex container that had been disinfected with the same alcohol to dry.

-once the slides were dry, I moved them to the prepared templates using sterile metal forceps.

-I prepared the CLEAN CONTROL tubes first

-after that I worked on 3 slides at a time (or one test group) to do the inoculation with the master culture, let the inoculum dry, apply disinfectant-dipped filter papers if necessary (for 5 minutes), let the residual disinfectant dry completely, then place each slide into its tube.

-I started with the INOCULATED CONTROL then did TEST DISINFECTANT 1 (Fantastik = sodium hypochlorite/bleach), TEST DISINFECTANT 2 (Mr. Clean = sodium hydroxide), then TEST DISINFECTANT 3 (Lysol = quaternary ammonium chloride)

-once all tubes were prepared (in the tube holder), I put them in the incubator

-it took until around 6:30pm to prepare all the tubes so they went in the incubator at this time

-I found several things:

-handling the glass slides with the metal forceps was difficult as the slides were too slippery. I accidentally dropped several back onto the template tray while trying to move them to their assigned test tubes.

-keeping the glass slides leaning upright for them to completely dry after they were put in the alcohol was also very hard

I recorded the following temperatures in my incubator during the second phase:

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 6:48PM | 32.8 | None |
| 8:46 PM | 31.8 | Closed lid slightly |
| 11:42 PM | 32.7 | None |
| 1:02AM | 33.3 | None |
| 10:05AM | 35.0 | None (removed to test) |

January 10th:

-10:30AM (16 hours of culture) - I removed all the culture tubes from the incubator and analyzed the liquid in each visually, by smell, and by the spectrophotometer.

**Qualitative (colour, texture):**

|  |  |
| --- | --- |
| **Tube** | **Observations** |
| Clean Control | All 3 tubes clear, smelled like original broth |
| Inoculated Control | All 3 tubes very cloudy, no big particles, smelled bad |
| Test disinfectant 1 | All 3 tubes clear, smelled like original broth. |
| Test disinfectant 2 | Tubes 1, 3 clear, smelled like original broth. Tube 2 slightly cloudy and smelled bad. |
| Test disinfectant 3 | All tubes clear, smelled like original broth. |

**Absorbance Readings:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trial | Clean Control | Inoculated  Control | Test Disinfectant 1  (sodium hypochlorite) | Test Disinfectant 2  (sodium hydroxide) | Test Disinfectant 3  (quat. ammonium chloride) |
| 1 | 0.028 | 0.660 | 0.022 | 0.028 | 0.029 |
| 2 | 0.025 | 0.724 | 0.021 | 0.416 | 0.030 |
| 3 | 0.021 | 0.787 | 0.020 | 0.023 | 0.026 |

-after completing the analysis, I realized that the spectrophotometer I was using had not stayed zeroed correctly and had been reading 0.025 too high. I suspect that this caused all my readings to be 0.025 too high. I plan to recheck the zeroing using the fresh broth cuvette between groups to make sure it still reads “0”.

-I believe that I that accidentally contaminated the one tube/slide in the second tube in the “Disinfectant 2” group as it had a very high reading compared to the rest. It could have gotten contaminated when I dropped the slide back onto the template tray while trying to transfer it to its test tube. I wonder whether there is a better way to hold onto the slides when moving them to avoid contaminating them.

**January 13-14, 2021**

-In the experiment run last week, one problem that occurred was that it was hard to disinfect the slides by dipping them in the alcohol. I wondered whether there was another way to disinfect them. I looked at whether sterile slides can be purchased but they are quite expensive and hard to find. A better choice would be asking the school lab to autoclave the slides. But it might be difficult to get them home without contaminating them. I did some research and found out that dry heat is another good way to sterilize glassware. The Centers for Disease Control in the USA also says that dry heat at 160 degrees Celsius (320 degrees Fahrenheit) for 120 minutes should be adequate.

-I did a trial of using dry heat from my home oven to sterilize some slides. First I placed 2 glass slides on baking parchment paper in a Pyrex glass baking dish. Then I put the dish in the oven and turned the oven on to 325 degrees Fahrenheit on convection. Once the temperature came up to this reading, I set the timer for 120 minutes. At the end of this time, I turned the oven off but left the dish inside with the door closed for another 60 minutes. Then I removed the dish and let it cool on the stovetop for another 10 minutes until the outside of the dish could be touched with bare hands. I removed each slide using the sterile metal forceps and placed it into its own culture tube containing 10mL of broth. I placed the 2 tubes in the incubator to culture at the usual temperature for 16 hours. I found the following when I analyzed the broth using the spectrophotometer on January 14:

-tube 1: -0.002

-tube 2: -0.007

-I concluded that there was no detectable growth of bacteria from either slide sterilized using this technique.

-an advantage to using this technique to sterilize the slides is that they can be prepared in a single pan and they do not have to be dried before use. Perhaps this technique for sterilization is easier to control and to make sure all slides are treated equally (compared to the alcohol dipping technique).

-I also found a silicone-tipped small kitchen tongs that I could disinfect to use to grip my slides when moving them to and from the template and tubes. Perhaps these tongs would reduce the risk of dropping the slides and contaminating them in my next trial.

-Audrey and I discussed repeating the experiment using what we have now learned. We will order more supplies to run another trial this weekend.

**January 15-17, 2021:**

Audrey and I did another run of the experiment using the same procedure. Each of us tested our original 3 different disinfectants. We also did the same initial phase, making initial cultures by swabbing our kitchen sinks and incubating 2 5 mL tubes of these swabs each before combining them into a master culture. The main difference with this run is that we are trying dry heat (as described above) to sterilize the slides before using them (instead of dipping them in isopropyl alcohol). Once the slides have been baked,we will use sterile gloves to move them to their templates. Then we will also focus on working on one group of 3 slides and completing all steps before moving on to another group rather than inoculating all 12 slides first before doing any other steps.

January 15th:

-10:13pm - placed my 2 tubes of 5mL broth produced from swabs of my kitchen sink and 1 control tube (saline-dipped swab only) into the incubator.

January 16th:

-overnight, I checked the temperature in my incubator and it ranged from 31.3 - 35.0 degrees Celsius.

-at 11:00am, I placed 15 glass slides in the oven to bake at 325 degrees Fahrenheit for 120 minutes, then rest in the oven until I was ready to use them for the second phase.

-at 2:15pm, I removed my 3 tubes from the incubator. The control broth was clear and the other tubes cloudy with no particles. The same as the week before, my mother met Audrey’s mother to combine (at 2:47pm) my 2 “Master Culture” tubes with Audrey’s 2 tubes to make a combine “Master Culture”. Each of us again got 10mL of this “Master Culture”.

-at 3:11pm, I used the spectrophotometer to test this combined master culture and my original control tube. I got the following results:

Master broth (combination of cultures from Audrey’s and my sinks): 0.525. It appeared cloudy with some dark particles.

Control broth (saline dipped swab): 0.000 (appeared clear)

-I then proceeded with the second stage of the experiment, making the slides and placing them in culture tubes. I took the slides out of the oven and took them to the experiment area. Using sterile gloves, I placed them on their templates (that had been cleaned with isopropyl alcohol). The rest of the procedure was the same as the week before. I did try using sterilized (submersed in isopropyl alcohol for 1 hour them flamed) silicone-tipped kitchen tongs to move a slide but it was too hard to get under the slide to lift it off the template so I went back to using the metal forceps. I did drop 2 slides (in “Test Disinfectant 1” group) back onto the general area of the template they came from when trying to move them to the tubes. All 15 slides were then placed in the incubator at 6:30pm.

I recorded the following temperatures in my incubator during the second phase:

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 6:35PM | 32.6 | None |
| 7:20 PM | 31.9 | Closed lid slightly |
| 12:25 AM | 32.6 | None |
| 9:26AM | 32.0 | None |
| 10:28AM | 31.2 | None (removed to test) |

January 17th:

-10:30AM (16 hours of culture) - I removed all the culture tubes from the incubator and analyzed the liquid in each visually, by smell, and by the spectrophotometer.

-I paid particular attention to calibrating the spectrophotometer to avoid the error in the “zero” that occurred last week. I also rechecked the “zeroing” between each measurement to ensure it had not changed. The spectrophotometer gave a reading of 0.001 for the cuvette with fresh broth when zeroed and 0.050 when the chamber of the analyzer was sitting empty waiting for another sample. Last week I found that the reading was 0.075 (or 0.025 too high) when the analyzer was resting with the empty chamber.

**Qualitative (colour, texture):**

|  |  |
| --- | --- |
| **Tube** | **Observations** |
| Clean Control | All smelled like original broth. Tubes 1 and 3 were very clear, tube 2 was very slightly cloudy. |
| Inoculated Control | All 3 tubes very cloudy, some particle in tubes 1 and 3, smelled very bad. |
| Test disinfectant 1 | All 3 tubes clear, smelled like original broth. |
| Test disinfectant 2 | All 3 tubes clear, smelled like original broth. |
| Test disinfectant 3 | All 3 tubes clear, smelled like original broth. |

**Absorbance Readings:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trial | Clean Control | Inoculated  Control | Test Disinfectant 1  (sodium hypochlorite) | Test Disinfectant 2  (sodium hydroxide) | Test Disinfectant 3  (quat. ammonium chloride) |
| 1 | -0.003 | 0.726 | 0.000 | -0.006 | -0.003 |
| 2 | 0.064 | 0.745 | 0.000 | -0.005 | 0.000 |
| 3 | -0.010 | 0.830 | -0.005 | -0.003 | -0.007 |

-I did have a slight bit of contamination in one “Clean Control” tube. However none of the slides treated with any of the 3 disinfectants had any growth at all.

-if my absorbance results from last week were all 0.025 too high, they are similar to the results I got today (except for the one outlier slide in “Test Disinfectant 2” group last week).

-Audrey and I decided that we probably have enough data on these 3 disinfectants but we should re-run the testing for her disinfectants next weekend. We may both use her 3 disinfectants in this upcoming run.

**January 22-24, 2021:**

Audrey and I did another run of the experiment using the same procedure except this time we both tested the 3 “natural” disinfectants. All steps were the same except we paid extra attention to sterilizing and disinfecting all equipment and work surfaces.

January 22nd:

-10:03pm - placed my 2 tubes of 5mL broth produced from swabs of my kitchen sink and 1 control tube (saline-dipped swab only) into the incubator.

January 23rd:

-overnight, I checked the temperature in my incubator and it ranged from 30.3 - 35.0 degrees Celsius.

-at 3:20pm, I used the spectrophotometer to test Audrey’s and my combined master culture and my original control tube. I got the following results:

Master broth (combination of cultures from Audrey’s and my sinks): 0.600. It appeared cloudy without particles.

Control broth (saline dipped swab): -0.003 (appeared clear)

Reading in empty chamber of spectrophotometer once zeroed: 0.054

-I then proceeded with the second stage of the experiment, making the slides and placing them in culture tubes using the same procedures as in the past with a few slight changes.

-the glass slides were baked in an oven using the same method as last week.

-one difference from the previous weeks is that 3 metal tin baking trays I use for a work surface for the templates and slides were also placed in the oven for the same amount of time as the slides to sterilize them with dry heat (to try to do everything possible to avoid any contamination). In previous weeks, I used these tins as the work surface for the templates but only used alcohol to sterilize them. Then these metal tins were also treated with 99% isopropyl alcohol before the templates were placed on them.

-as I did not have any sterile gloves this week, I used flamed metal forceps to move the slides from the baking dish to the templates.

-the 3 disinfectants I used this time were Seventh Generation (thymol), Method (citric acid), and Natura (hydrogen peroxide).

-I found that the filter papers dipped in all 3 of these disinfectants acted similarly to the other 3 disinfectants I tested in my other 2 runs.

-I did note that the Seventh Generation and Natura disinfectant residuals tended to bead up and take longer to dry on the slides after the filter papers were removed than the other 4 disinfectants tested.

-a few of the slides treated with disinfectants stuck to the templates (when the cleaner dried) and were hard to move to the tubes smoothly using the metal forceps.

-I prepared all slides and tubes and put them in the incubator by 7:00PM

I recorded the following temperatures in my incubator during the second phase:

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 7:00PM | 33.7 | None |
| 7:50 PM | 33.1 | None |
| 10:21PM | 31.3 | Closed lid slightly |
| 10:59PM | 32.3 | None |
| 12:42AM | 32.1 | Closed lid slightly |
| 9:16AM | 33.7 | None |
| 11:10AM | 33.6 | None |

January 24th:

-11:10AM (16 hours of culture) - I removed all the culture tubes from the incubator and analyzed the liquid in each visually, by smell, and by the spectrophotometer.

-my spectrophotometer, once zeroed against fresh broth in a cuvette, gave the following readings:

-with fresh broth in cuvette in chamber: 0.000

-with empty chamber: 0.057

**Qualitative (colour, texture):**

|  |  |
| --- | --- |
| **Tube** | **Observations** |
| Clean Control | All 3 tubes clear, smelled like original broth. |
| Inoculated Control | All 3 tubes very cloudy and very smelly but had no particles. |
| Test disinfectant 1 | All 3 tubes clear, smelled like original broth. |
| Test disinfectant 2 | Tubes 1 and 3 were clear, smelled like original broth. Tube 2 was cloudy with a slight odour. |
| Test disinfectant 3 | All 3 tubes clear, smelled like original broth. |

**Absorbance Readings:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trial | Clean Control | Inoculated  Control | Test Disinfectant 1  (thymol) | Test Disinfectant 2  (citric acid) | Test Disinfectant 3  (hydrogen peroxide) |
| 1 | -0.004 | 0.784 | 0.002 | 0.006 | -0.020 |
| 2 | -0.005 | 0.695 | 0.000 | 0.550 | 0.004 |
| 3 | 0.000 | 0.684 | 0.004 | -0.004 | -0.010 |

-I did not have any growth in the Clean Controls and Test Disinfectants 1 and 3. But I did have good growth in the untreated Inoculated Control group. In 2 of my tubes in the Test Disinfectant 2 group, I also had no growth. However, I did have an outlier reading in tube #2. I wonder if this outlier could have been due to accidental contamination when trying to move the slide to the tube with the metal forceps.

Audrey and I plan now to review our results with Ms. Grelowski to discuss:

1. Do we have enough data or do we need to do any more trials?
2. Should we pick one trial for each set of disinfectants to use (mine from Jan. 17th and Audrey’s from Jan. 24th)? Or should we use the 2 best for each (mine from Jan.10th and 17th and Audrey’s plus mine from Jan. 24th) even though we had some less than ideal trials?

After discussion with Ms. Grewloski, we have decided to combine the 2 best runs for each group of disinfectants (traditional and eco-friendly) to get a total of 6 test slides for each group. Ms. Grewloski thinks we have done enough testing now.

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# Audrey:

# **November 21, 2020**

Today we called and discussed the project. We have an appointment with Dr. Garcia Diaz to talk about the project. I want to discuss the details of the procedure today.

Make 2 graphs to record results: 1 for eco friendly+1 for chemical

Variables:

* Independent
  + Types of disinfectants
* Dependent
  + How much bacteria is left after experiment
* Controlled
  + Location?
    - One location

Microbial disks

Lawn of bacteria and disks dipped in disinfectant

Can't control amount of disinfectant

Slide smear: Smear with bacteria

Disinfectant dip for several minutes, let dry

Fresh broth, let dry

Turbidity

How to clean microscope slides:

Leave in ethanol and flame

Make a Timeline/ Plan

Think about when to do bacterial inoculation

Things to research:

Credible sources

How disinfectants kill bacteria

How to compare

Natural vs industrial disinfectants

Environmental/economical concerns

Types of agar (Dalynn Biologicals NE; ask for microbiologist)(Nutrient, soy, blood?)

Types of bacteria

Which bacterial types to use

Bacteria vs virus

Testing antibacterial activity (examples and demonstrations)

Methods of growing bacteria

Disinfectants to use

Types of broths for bacterial growth(Bottle????????????)

Growing a bacterial lawn

Best medium (Agar+Broth?)

Backup plan

WHAT IS TUBE MEDIA

Time it takes to develop

Time it takes to disinfect

Dilution

How to transfer tubes

Locations to swab (shoes, school floor, locker, house floor, phone, hands)

### **November 22, 2020**

I looked at the sources that Dr. Garcia Diaz gave us yesterday. The video (<https://www.youtube.com/watch?v=bHq_LFlwdjs>) was helpful in explaining the process of the experiment. Grace and I called and talked about what locations we should test and what methods we could use. We have decided on the liquid method(experiment), the lawn method(bacterial culture), bathroom, shower, kitchen, hands, bottom of shoes, phone(locations for testing). We are still trying to figure out whether we should test at one location (the school), two locations (our houses or the school plus one of our houses), or all three (school and our houses). We are writing up our procedure.

**Procedure Summary from video, with modifications to suit project:**

Part 1

1. Put on fitted, non-latex gloves
2. Take sterile swab stick out of package (DO NOT TOUCH TIP OF STICK)
3. Swab crosshatch with sterile swab sticks of the following:
   1. Shower floor
   2. Phones
   3. Kitchen counter
   4. Bottom of shoes
   5. Palms
4. Put swab in solution for storage
5. Put a zigzag pattern with the sl.wab on an agar plate
   1. What kind of method?
      * + 1. zigzag/quadrant
6. Incubate 37 degrees centigrade 24-48 hours
7. If bacteria survived, take (specified amount) of bacteria and culture in broth to ensure that it will survive there
8. Record Results

Part 2 (actual experiment)

1. Culture bacteria on agar petri dish
2. Take 1/10 microlitre of bacteria and place in broth
3. Culture
4. Sterilize microscope slides
   1. Dip in ethanol
   2. Flame
5. Smear one kind of bacteria on the same amount of slides as disinfectants
6. Let air dry
7. Dip each sample in a separate disinfectant for 5 minutes, ensure smear is submerged
8. Let air dry
9. Dip each sample in it's own unaltered broth for 5 minutes, ensure smear is submerged
10. Let air dry

\*\*\*\*\*\*\*\*This isn't the actual procedure. I made this in case we wanted to use the procedure from the video\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

### **November 25, 2020**

I met up with Dr. Garcia Diaz in the morning today, but I forgot to go back up to the science lab to get my supplies. Whoops… Oh well. I'll go back tomorrow. She showed me how to collect the bacteria from a surface and put it in an agar plate or in the broth. I think I'll show Grace how to do it tomorrow, but I'll have to pretend there's a blowtorch and mime all of the other stuff. Grace and I tested out Hangouts as a viable option for communication, however, the audio keeps cutting in and out. A better way to describe it would be that the audio starts out normal, but slowly decreases in volume until it's silent, and then jumps back to being normal, which then sounds really loud.

**November 26, 2020**

Today I feel tired.

**November 28, 2020**

**Documentation of Nov. 25, 2020 Lab Procedures with Dr. Garcia Diaz taken from the other Google Doc we had(CORRECTION: We now have three Google Docs for Science Fair, and the one that I originally wrote this on was called "Science Fair Planning Disinfect):**

READ ALL INSTRUCTIONS BEFORE PROCEEDING TO USE

This is for the control group. This is not for the actual test, then there would be added steps.

1. For the agar transfer
   1. Label petri dish with agar on the bottom with
      1. Month/Day/Year ex. 11/26/2020
   2. Take the sterile swab, put it in the saline, and move your hand in a clockwise motion six times without squeezing the saline out
   3. Cap the saline to keep sterile, then wipe the surface with the swab until all sides of the swab are covered
   4. Take the agar plate(Make sure you left it upside down to keep dust out), open it, and in a wide zigzag motion apply the bacteria across the full plate, and turn about 45 degrees
      1. **PRESS GENTLY! THE BACTERIA WILL GO ON! AGAR IS VERY DELICATE AND YOU DO NOT WANT TO DAMAGE IT!**
      2. **I literally cannot emphasize the point above enough.**
   5. Repeat process 3 more times for good measure
   6. Cover and flip upside down to reduce dust contamination
   7. Put in incubator/ place where it stays a steady temperature and won't be disturbed
   8. Done!
2. For the broth transfer
   1. Label tubes with the same label as the corresponding petri dish
   2. Open agar plate with gloves. Safety before… anything, really
   3. Take a sterile swab and repeat the same swabbing process but be **very gentle with the agar**.
   4. Dip and swirl in a falcon tube with 5 ml of broth to ensure even saturation
   5. Cap loosely so that the cap won't fall off if the tube is shaken but it's not tight
   6. Put falcon tubes in agitator and ensure it's at (the speed it's supposed to be at)
   7. Leave for 24-48 hrs
   8. Done.

**November 29**

We built a homemade incubator yesterday (November 28). We had adjusted the lightbulb several times to try to keep it at a steady temperature between 36-38 degrees centigrade. We monitored it for several hours to get some practice and adjusted the styrofoam lid to get to the target temperature range:

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 9:45 AM | 34.0 | Lightbulb at original position |
| 9:50 AM | 37.5 | None |
| 10:08 AM | 37.6 | None |
| 10:15 AM | 37.2 | None |
| 10:33 AM | 36.9 | None |
| 11:44 AM | 36.5 | Pushed bulb in by ~1cm |
| 12:01 PM | 37.0 | None |
| 12:17 PM | 37.1 | None |
| 12:47 PM | 36.7 | Pushed bulb in by ~0.5cm |
| 1:08 PM | 37.8 | None |

Then Grace said to put it at 32-35 degrees, so we adjusted accordingly.

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 1:28 PM | 37.8 | Pulled bulb out by ~1cm |
| 2:00 PM | 36.4 |  |
| 3:50 PM | 36.4 |  |
| 4:07 PM | 36.2 | Raised the lid by ~1cm |
| Time | Temperature in /degrees C | Action Taken |
| 4:27 PM | 35.6 | None |
| 4:45 PM | 35.2 | None |
| 5:02 PM | 34.6 | None |
| 5:32 PM | 34.2 | Moved the lid down by ~0.5cm |
| 5:49 PM | 34.6 | None |
| 7:30 PM | 34.5 | None |

We moved the temperature probe to the bottom because that's where the agar plates would be. We also propped the lid up with popsicle sticks to ensure that it wouldn't overheat. I was going to send her a video about how Dr. Garcia Diaz showed me how to collect agar plate samples, but then she did it before I finished the video. I finished the standard for the bacteria and I now know that you should roll the swab on the bacteria, not swipe it. I also learned that the bacteria smells very gross, and to always be prepared for the worst when you open the petri dish.

Procedure to make the incubator:

* 1. Place a 1.5 - 2 cubic feet styrofoam box on a sturdy surface
  2. Plug in light bulb into the light bulb socket and cord that will reach a power source
  3. Lower thermometer sensor wire from one side and onto the bottom of the styrofoam box, leaving the temperature gauge screen sitting on the surface outside the box. Use duct tape to tape the wire of the thermometer to the side and bottom of the inside of the box, taking care not to cover the sensor
  4. Rest the cord attached to the lightbulb over the top edge of the box, with the lightbulb positioned near and over the centre of the box.
  5. Tape the cord over the edge and down the inside of the box
  6. Put the cover of the box on top
  7. On the opposite side to where the lightbulb cord rests, tape the outside of the box to the lid to act as a hinge
  8. On the edge where the cord is in the rim, insert two popsicle sticks into the edge, about two inches away from each side of the cord
  9. Turn the lightbulb on
  10. Set the open side of the lid on the tips of the popsicle sticks. The lid of the box can be opened at varying degrees by raising or lowering the popsicle sticks to adjust temperature inside the box

Today I also practiced growing a culture at home

1. Turn on the homemade incubator to get temperature to about 35 Degrees C:

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 7:32 PM | 31.3 | None |
| 8:13 PM | 33.8 | None |
| 8:44 PM | 34.9 | Open the lid ~2cm |
| 9:05 PM | 33.8 | None |
| 9:20 PM | 34.2 | None |
| 9: 41 PM | 33.6 | Closed the lid ~1cm |
| 10:35 PM | 34.7 | None |
| 10:48 PM | 34.6 | None |

1. Let agar sit to room temp.
2. Dipped swab in saline and swirl a few times
3. Swabbed my left palm
4. Transferred bacteria to agar plate using zigzag on entire plate surface, rotated 45 degrees each time and repeat 3 more times
5. Put the lid back on and taped the lid on
6. Flipped the agar plate upside-down
7. Labelled plate with time, date, location bacteria was from
8. Put into homemade incubator

Transfer of Shoe Bacteria to Broth from Agar:

1. Flamed broth cap and tube cap with BBQ lighter
2. Transferred 5 mls of broth to falcon tube before capping very quickly
3. Took 6 spins of sterile saline on sterile swab
4. Swabbed the large splotch in the agar plate
5. Rolled it around until swab was coated in beige chunks
6. Put swab in broth and swirled it around until the swab had no more chunks in it
7. Capped the broth loosely before incubating

**November 30**

Observations about yesterday's trials

Hand Swab Agar:

There is condensation on the lid of the plate with my hand swab but it didn't touch the agar. The agar had tiny scattered colonies today, about a millimeter large in diameter each and was taken out of the incubator at 7:45 am to refrigerate.

Shoe Broth Culture from Agar:

Yesterday I swabbed the agar plate with my shoe swabbed in it and transferred part of a larger looking "colony" into a broth. Today we found that the broth had a large ribbony substance in it which Dr. Garcia Diaz later told us that she highly suspected it was fungus. The strange substance occupied about one third of the broth. This most likely happened because I swabbed the large splotch in the agar plate rather than the small bacterial colonies around it.

The Control:

1. I did the control with just the saline today.
2. I labelled both of the tubes we were using today as 'Control' and 'Basement TV Room Door Handle', and underneath wrote 11/30/20 (date)
3. My mom helped me flame the broth bottle rim and the falcon tube rims so that the broth could stay sterile while I poured it into the tubes
4. I poured 5 ml of broth into each tube
5. Opened the cap of saline and swirled swab inside 6 times before capping quickly to reduce contamination
6. Swirled swab with saline inside of control tube

The Basement Door Handle:

Because of the 'potentially fungus incident', I thought it best to redo the trial on a place where fungus couldn't grow. I decided on the door handle to the basement exercise/TV room because it was an area where my mom hadn't sanitized yet and made of metal, preventing fungus from growing on it.

After I finished the steps above:

1. I brought the tubes downstairs to swab
2. I spun the swab in the saline six times, capped the saline tightly, swabbed the handle while rotating the swab
3. Spin swab in broth in tube labelled"Basement TV room Door Handle"
4. Capped falcon tube loosely
5. Put tubes in incubator (8:56 PM, Temperature in incubator at 34.4 Degrees C)

**December 1**

Calibrating the Spectrophotometer:

Tried out the spectrophotometer today. It will be used to measure the amount of turbidity of different bacterial culture samples after the culture in the broth has been treated with different disinfectants. Dr. Garcia-Diaz let us borrow one from the school, along with instructions on how to use it.

1. Connect the spectrophotometer to the LabQuest machine with the USB cable. Connect the LabQuest to a power source
2. Turn on by pressing the small button on the LabQuest
3. Press "Sensor" menu
4. Press "Calibrate"
5. Choose "USB Spectrometer"
6. While it's warming up, pour fresh broth into a cuvette (Only touch the ridged sides and not the smooth sides)
7. Place cuvette into the spectrometer, the smooth sides facing the light beam
8. Press "Finish Calibration" and "OK"
9. Take out the cuvette
10. Press "Mode" box on the right hand side
11. Choose "Time-based" and press "OK"
12. Tap the red box in the middle of the screen to change the wavelength to 600
13. Press "OK"
14. Take out the "Control" broth and "Basement TV Room Door" culture in broth from the incubator and let them go to room temperature
15. Gently shake the tubes in case any bacteria has settled on the bottom
16. Pour the Control broth into a new cuvette
17. Place into the spectrometer to measure turbidity and record the number
18. Pour broth with basement door handle bacteria into another cuvette
19. Place into the spectrometer to measure and record the number

**Observations:**

**Quantitative:**

|  |  |
| --- | --- |
| Sample | Spectrometer Reading |
| Control | -0.007 |
| Basement Door Handle | 0.892 |

**Qualitative:**

The Control is very clear and I can see all the way through the tube. The colour is yellow. There is no turbidity at all. The Basement Door Handle broth is very turbid and cloudy. There are bits and clumps in it too. The colour is lighter than the Control. I think the Control has a negative number because it has been diluted with the saline from when I dipped the swab in to wet it.

(\*\*Have to find out what a negative number means\*\*)

1. Put the Control and Basement Door Handle broth into the fridge to save them.

**December 5**

Let's try with some disinfectants:

1. Turn on the incubator to let it warm up to about 35 degrees C. Instead of pulling bulb in and out to control temperature. I will try to lift the lid up and down a bit at a time to adjust the temperature.

|  |  |  |
| --- | --- | --- |
| Time | Temperature in Degrees C | Action Taken |
| 11:45 AM | 34.6 | None |
| 12:10 PM | 36.0 | Opened lid by ~2cm |
| 12:27 PM | 34.0 | None |
| 12: 40 |  |  |

**January 8, 2021**

**Experiment #1**

**Materials (for each experimenter):**

* 2 sterile cotton swabs
* 250mL Tryptic Soy broth (need 10ml/tube x 20 tubes plus a bit extra for blank calibrating cuvettes). Keep in refrigerator at 4-8 degrees Celsius.
* 20 Falcon plastic 50mL sterile test tubes
* Test tube holder for 18 test tubes (styrofoam block)
* 1x1mL vial of sterile normal saline
* Homemade incubator – (GE 40w 330 lumen appliance light bulb, extension cord, light bulb socket with plug, Taylor Indoor/Outdoor Wired thermometer, styrofoam cooler box with lid (our cooler is 38L or 1.34 cubic feet, ?yours is 1.5-2 cubic feet), duct tape, scissors, ruler)
* BBQ lighter
* 18 glass slides
* 3 paper templates, each with five rectangles representing the glass slides, with a 1cm x 2cm portion drawn at one end of each slide pictures
* 3 plastic paper protectors
* Three different disinfectants (30 ml each)
* 4 x 125 mL mason jars
* SpectroVis Plus spectrophotometer (Vernier)
* LabQuest2 (Vernier)
* 18 Cuvettes for measuring absorbance of samples
* No. 4 coffee filters - cut into 12 pieces each measuring 2.5x4cm
* Containers for safe disposal of materials
* Isopropyl alcohol or ethanol disinfecting spray
* Alcohol hand sanitizer

**Procedures:**

**Incubator:**

* 1. Place a 1.5 - 2 cubic feet styrofoam box on a sturdy surface
  2. Plug in light bulb into the light bulb socket and cord that will reach a power source
  3. Lower thermometer sensor wire from one side and onto the bottom of the styrofoam box, leaving the temperature gauge screen sitting on the surface outside the box. Use duct tape to tape down the wire on the inside wall of the box, but don't cover the sensor
  4. Let the cord rest on the edge of the box with the light bulb hanging inside
  5. Tape the cord over the edge and down the inside of the box
  6. Put the cover of the box on top
  7. On the opposite side to where the lightbulb cord rests, tape the outside of the box to the lid to act as a hinge
  8. On the edge where the cord is in the rim, put two popsicle sticks into the edge, about two inches away from each side of the cord
  9. Turn the lightbulb on
  10. Set the open side of the lid on the tips of the popsicle sticks. The lid of the box can be opened at at different amounts by raising or lowering the popsicle sticks to adjust temperature inside the box

1. **To Grow a Master Source of Bacteria in Broth:**
   1. Wash hands with soap before starting. Clean and prepare all work surfaces
   2. Label one of the test tubes with the date and the word "Control"
   3. Label 2 test tubes with the date and the word “Master Source”
   4. Place all three test tubes in the test tube holders and open the caps
   5. Remove the Tryptic Soy broth from the refrigerator. Open the cap
   6. Using the flame of a small BBQ lighter, quickly flame the edges of the broth bottle to sterilize the rim
   7. Pour 5mL of broth into each of the test tubes. Let the broth in the tubes sit for at least 5 minutes to allow the broth to warm up to room temperature
   8. Recap the broth bottle and return it to the refrigerator
   9. **For the Control:**
      1. Open the cap of a small normal saline vial.
      2. Take a sterile swab out of the package, taking care not to touch any other swabs in the package. Only touch the wooden part of the swab so you don't contaminate it
      3. Dip the tip into the normal saline
      4. Swirl the tip in the saline a few times and take out the swab
      5. Put the saline-dipped swab into the broth of the test tube labeled with the date and "Control"
      6. Swirl about 6 times and take out the swab
      7. Cap the tube loosely and discard the used swab
      8. Mark the time on the tube
   10. **To Culture Bacteria in Broth (the Master Source):**
       1. Take a new sterile swab and dip the tip into the original sterile saline vial
       2. Swirl the tip in the saline a few times and take out the swab
       3. Rub the swab on a dirty surface (In our experiment we rubbed around the rim inside our kitchen sink)
       4. Insert the swab into the broth of the test tube labeled with the date and "Master Broth".
       5. Swirl about 6 times and take out the swab.
       6. Cap the test tube loosely and discard the used swab.
       7. Mark the time on the tube.
       8. Repeat steps "j" to make 2 x 5ml Master Broth cultures.
   11. Place the three tubes in the tube holder, then place the rack or holder onto the bottom of the styrofoam box incubator, with the temperature preheated to 31-35 degrees Celsius.
   12. When the cover of the box is lifted to allow the set up to be placed inside, temperature may drop slightly. Check the temperature a few times before going to bed to make sure it is steady around 33 degrees C.
   13. Incubate for 16 hours
   14. After incubation is done, take out the Control and two Master Broth tubes
       1. Combine the two Master Broth tubes from each experimenter (4 x 5mL Master Source tubes) to make one combined tube
       2. Divide up the one tube into 2 x 10ml so the broth culture used for the whole experiment is the same for both experimenters
   15. Turn on and calibrate the Spectrophotometer
   16. Use fresh Tryptic Soy broth as the "Blank" to finish the calibration
   17. Gently shake the broth in the culture tube immediately before testing so the turbidity is evenly distributed
   18. Pour from the “Control” tube into the test cuvette until the liquid comes up in the cuvette higher than the upper lip of the test slot, approximately three-quarters of the way
   19. Measure and record absorbance for the Control.
   20. Repeat the same procedure, using a new test cuvette, for the broth from the 10ml Master Broth tube
   21. Do not discard the remaining Master Source as it will be needed for the rest of the experiment
2. **Testing Disinfectants in Broth Culture**
   1. There will be 5 test groups with 3 slides (for 3 Trials) in each. These test groups are as follows:
      1. Negative control - no bacterial smear, no disinfectant treatment
         1. Dr. Garcis-Diaz says the Negative control is just a clean glass slide to show there is no contamination
      2. Inoculated control - bacterial smear, no disinfectant treatment
      3. Test disinfectant 1
      4. Test disinfectant 2
      5. Test disinfectant 3
   2. Clean a work surface with 70% alcohol.
   3. Prepare and label 15 culture tubes with the date and test groups
   4. Place the tubes in the tube holder and remove the caps
   5. Remove the Tryptic Soy broth from the refrigerator so it can warm up
   6. Open the cap
   7. Using the flame of a small BBQ lighter, quickly flame the edges of the broth bottle to sterilize the rim
   8. Pour 10ml of fresh broth into each test tube and let the broth come up to room temperature
   9. Prepare the slide templates (One template per trial):
      1. For each of the 3 paper slides templates, label each position with "Negative Control", "Inoculated Control", "Test disinfectant 1", "Test disinfectant 2", and "Test disinfectant 3". Insert the templates into plastic protectors. Clean the surface of the plastic protector with 70-99% alcohol and let them dry.
3. In a 125mL glass jar, fill with about 50mL alcohol cleaner to clean the slides
4. To clean the slides, dip each one into the alcohol for about 10 seconds, then prop each slide up in an alcohol-cleaned container to let dry completely over several minutes
5. Place slides on each of the labeled paper templates
6. For the Smear:
   1. Gently shake the Master Broth for a few seconds so the broth looks consistently murky throughout
   2. Open the cap of the culture
   3. Use a micropipette to draw up 5microlitre of broth
   4. Push out and distribute the contents of the micropipette tip onto one of the glass slides within the 1x2cm area shown in the template
   5. Repeat steps *ii to iv* for all the slides except the Negative Control
   6. Let the slides dry for 4 - 5 minutes
   7. Place the 3 slides from the Inoculated Control group directly into their appropriate culture tubes so that the test end of the slides are submerged and covered by broth. Put the lids on the culture tubes loosely and put the tubes back into the holder
7. **To Expose Slides to Disinfectants**
   1. Cut nine 2.5 x 4cm pieces of filter papers
   2. Label 3 x 125ml mason jar containers with "Test disinfectant 1", or "...2" or "...3"
   3. Pour approximately 20 ml of the disinfectant into each of the correct jars
   4. Flame a metal forceps then use it to transfer 3 pieces of the cut filter papers into the each container of disinfectant
   5. Leave the pieces to sit in the disinfectant for at least one minute.
   6. Pick up a piece of the filter paper from the disinfectant with the sterile forceps and let access drops to drip off
   7. Place the disinfectant-soaked filter paper on top of the end of the corresponding slide where the dried smear is. The paper should cover the whole width of the slide (2.5cm) and come up over 4cm at the end of the slide to completely cover the inoculated area
   8. Repeat this same procedure, flaming the tip of the forceps before each paper transfer, until all the slides have the correct filter paper on
   9. Let the papers sit on the 3 slides for a total of 5 minutes
   10. Flame the forcep tip, remove each filter paper from the slides and discard the papers.
   11. Flame the forceps again and use them to pick up the slide by the clean end. Place the slides immediately into the appropriately labelled tubes with broth, with the inoculated end submerged in the broth.
   12. Place the cap loosely on the tubes
   13. Place the tube holder with the 15 culture tubes into the prepared incubator and incubate for 16 hours, checking several times that the temperature in the incubator stays at 31-35 degrees Celsius (as close to 33 degrees as possible)
   14. At 16 hours, the appearance, smell, and turbidity will be analyzed for each culture tube
8. **Measure Turbidity of the New Cultures After Exposure to Disinfectants**
   1. Take out the test tubes from the incubator
   2. To review, there should be 15 test tubes with one slide each inside:
      1. Negative Control: cleaned, unsmeared, untreated
      2. Control: cleaned, smeared
      3. 3 tubes each with a slide treated with three different disinfectants and labeled correspondingly
   3. Following instructions to measure the absorbance, make sure the Labquest2 machine is in the Time-based mode, set to wavelength 600nm.
   4. Gently agitate each test tube to even out the turbidity in the broths
   5. Add each of the broth into a separate cuvette, about 3mL or three-quarters full
   6. Clean the plastic cover of the three templates and place the cuvettes in the appropriate spot
   7. Place each cuvette into the testing slot and record the absorbance
   8. Repeat with the other cuvettes

\*\*\*\*\*\*\*\*Please see above for the Procedure\*\*\*\*\*\*\*\*

Today I made a two-part master broth for the testing and a control. I took the samples from the rim under the sink. That place is DISGUSTING. The samples and Control were taken at 10:10 pm and put into the incubator at 10:16 pm at 33.9 degrees centigrade. We followed the procedure in one of the other Science Fair documents we made.

I learned how to use a micropipette, change the settings, change the tip, and suck up liquid. I learned that you push the micropipette halfway down, put the tip into the liquid, and let go to suck up liquid and to press full way down to eject liquid.

**January 9, 2021**

Today is experiment day. I checked the temperature of the incubator to make sure it is still between 32-35 degrees celsius:

10:30am: 34.8

12:54pm: 34.0

1:12pm: 33.9

1:53pm: 34.2

2:22pm: 33.8

The test tubes were taken out of the incubator at about 2:22pm. Since Grace and I are partners, we have to share our broths. Our moms are meeting up to mix the four tubes of Master Broths together. Then we each get 10ml to use for our experiments at our own home.

**Observations:**

**Qualitative:**

(Before our parents meet up to mix the broths together) The Master Broth #1 is more turbid, darker and more opaque than Master Broth #2. The control is still really clear and yellowy, which is good.

After my dad came home with the 10ml of master broth:

**Qualitative:**

The one test tube of 10 ml of Master Broth is very turbid. The colour is dark yellow. There are a few tiny floating bits when I shake it.

**Quantitative:**

I calibrated the spectrophotometer. I used fresh tryptic soy broth as the "dark" sample.

|  |  |
| --- | --- |
| Sample | Spectrometer Reading |
| Control | -0.008 |
| Master Broth (mixed) | 0.562 |

For the main experiment:

In this experiment, I will be putting bacterial culture in broth onto glass slides, then the slides will be dipped into jars of different disinfectants for 3 minutes. Then the slides will be placed into 50ml plastic Falcon tubes filled with 10ml of fresh tryptic soy broth each. The tubes will be put into the incubator for 16 hours to see if bacteria will grow. See above for the procedure.

**January 10**

9:38AM: 32.3 degrees C, test tube holder is taken out of the incubator

We left the spectrophotometer turned on overnight so we didn't recalibrate. I put a piece of clean glass slide over the top of the slot where the cuvette will go in so dusk wouldn't get inside overnight.

**Observations**

**Qualitative (colour, texture, smell):**

|  |  |
| --- | --- |
| Tube | Observations |
| In general | Varied slightly in colour with yellow and a small bit of orange, original broth colour yellow. Some lumps in some of the broths, and in others there were no lumps. |
| Clean Control | Relatively clear |
| Control | Very opaque. Smells awful! |
| Test disinfectant 1 | Varied in transparency (trial 1 clear, trials 2 and 3 opaque)  The tubes with growth both smell terrible! The clear one smells like the clean control, just broth-smelling |
| Test disinfectant 2 | Varied in transparency (trials 1 and 2 opaque, trial 3 clear)  The tubes with growth smell bad. The clear one smells like just broth |
| Test disinfectant 3 | Varied in transparency (trials 1 and 2 clear, trial 3 opaque)  The clear ones smell like the broth. The one with growth smells bad like the other ones that are turbid |

**Quantitative:**

Time we took test tube holder out of the incubator:9:38 AM

Temperature of incubator this morning: 32.2 degrees Celsius

Absorbance Readings:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trial | Clean Control | Control | Test Disinfectant 1 | Test Disinfectant 2 | Test Disinfectant 3 |
| 1 | -0.002 | 0.546 | 0.000 | 0.474 | -0.001 |
| 2 | -0.001 | 0.643 | 0.468 | 0.400 | 0.006 |
| 3 | -0.001 | 0.652 | 0.530 | 0.000 | 0.450 |

We talked to Grace's mom. She said their results were very consistent. But it turned out we were not doing the same procedure. They were doing something different from the procedure we were following. So I don't think we can count this trial. This is also our first one so we can't expect it to be perfect right away.

**Analysis:**

My mom talked to Grace's mom, who said they didn't smear all 12 slides at once like the procedure that I followed. I found that I couldn't keep up with setting the timer for all the slides one after another. The first ones I smeared started to dry up before I finished smearing the last ones. Using the forceps to lift the filter papers from the jars was very clumsy. They kept getting stuck together so the timing was not the same for all the slides. My mom was trying to flame the forcep tips in between because the BBQ lighter was hard to use. Then the procedure said to use forceps to pick up the glass slides which was impossible because they kept slipping. Instead, I sanitized my hands in between picking up the slides and putting each one into the appropriate test tubes.

I believe my results are inconsistent because the procedure didn't work the way I thought. After my mom talked to Grace's mom, Grace's mom said she would get Grace to update the different parts in the procedure to show what they actually did. I learned that I have to be more careful with keeping everything clean to avoid contamination. I also learned that I cannot do 12 slides all at once! It didn't make any sense!

**January 15**

**Experiment # 2**

There are some changes to the procedure. Mainly, the slides need to be baked now because Grace thinks hers were contaminated. Ours were definitely contaminated. We changed to this instead of cleaning the slides in a jar of alcohol:

* 1. - or use dry heat sterilization: place a piece of baking parchment paper in the bottom of a 9x13” Pyrex baking pan. Place 15 glass slides in a single layer on the parchment paper in the pan. Place the pan in the oven. Turn the oven onto “convection” at 325 degree Fahrenheit. Once the temperature reaches this level, set the timer for 120 minutes. After this time has passed, leave the pan inside the oven the door closed and let the slides cool slowly - for 60 minutes. Then remove the pan from the oven with oven gloves (as it will still be very warm). Be careful not to touch the inside of the pan. Set the pan on the top of the stove for another 10 minutes so the slides cool adequately. Then the slides are ready to place on the template (use sterile technique to move them to the template).

Grace was also using 99% isopropyl alcohol to clean her work surfaces and slides so we went out to get the 99% as well to be consistent in our procedures.

This time, Grace and I discussed and agreed on the procedure before beginning because I think it is very important that we do exactly the same procedure to be consistent because we already can't work in a lab together. Grace changed this part:

**Do one trial or template (group of 3 slides) at a time:**

* 1. **For the Smear** (On each template, use 4 out of the 5 cleaned slides only, as one slide will be the Clean Control - what does this mean?):
     1. Using the sterilized forceps or tongs, move the 3 slides in the Clean Control group to their appropriate culture tubes as they will not be inoculated with bacteria. Put the slides on the culture tubes loosely and arrange in the tube holder.

10:02pm: 32.5 degrees C, Control and 2 Master Broths are in the incubator.

**January 16**

2:07pm: took out of incubator

**Observations:**

**Qualitative:**

MS#1 is slightly cloudy but not that opaque, can kind of see finger through broth

MS#2 - slightly cloudier than MS#1 but very similar

Control - clear, yellow tint (all have yellow tint)

Our moms met up again to mix the Master Broths together then split them up into one 10ml Master Broth.

**Quantitative:**

|  |  |
| --- | --- |
| Sample | Spectrometer Reading |
| Control | -0.008 |
| Master Broth (mixed) | 0.537 |

**January 17**

**Observations**

**Qualitative:**

|  |  |
| --- | --- |
| Type of tube | Observations |
| General | Yellow color |
| Clean control | The first two clean controls were clear, but the third one was contaminated |
| Inoculated control | Very opaque, could not see anything on the other side of broth |
| Test Experiment 1 | Relatively opaque, but slightly less than inoculated, could see slight coloration on other side (e.x., I put my finger on one side and saw a very slight tint of my skin color) |
| Test Experiment 2 | First one about as opaque as the first disinfectant, but second two were about as clear as the clean controls |
| Test Experiment 3 | Slightly more clear than disinfectant 1 (could see slight outline of finger on other side) |

**Quantitative:**

Time we took test tube holder out of the incubator: 8:51 AM

Temperature of incubator this morning: 33.3 degrees Celsius

Absorbance Readings:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trial | Clean Control | Control | Test Disinfectant 1 | Test Disinfectant 2 | Test Disinfectant 3 |
| 1 | 0.000 | 0.558 | 0.533 | 0.478 | -0.573 |
| 2 | 0.001 | 0.533 | 0.467 | -0.001 | 0.343 |
| 3 | 0.351 | 0.629 | 0.473 | -0.002 | 0.485 |

**Analysis:**

I had even more contamination this time. I know it's not the disinfectant not being effective, because my results from last time showed it can kill bacteria very well in some of the tubes.

1. I cleaned the work surfaces and materials by spraying with the 99% alcohol, but it dried up very quickly. I kept spraying multiple times and it would just dry up. I can't be sure I was able to clean the work surfaces properly before I set up the rest of the experiment. I have to ask Grace how she managed to clean this way.
2. For some reason, the tubes seemed smaller this time, so the glass slides did not fall right into the broth. I had to sanitize my finger then push it down into the broth before capping them. I am thinking maybe two different companies made the different batches of Falcon tubes.
3. Something else I noticed was that when I put the 7th Generation filter paper on the slide, it kept on bubbling up, so I had to keep pushing it back down with tweezers to get it to maintain contact with the slide.
4. The Natura also dried quicker than the 5 minutes so the corners of the filter paper started to lift up.
5. After talking to Grace, Grace was actually also wiping her counter down with Lysol, then soaking layers of paper towels in the isopropyl alcohol to put inside the aluminum liners to sterilize them for several minutes. I didn't know this was part of the procedure so didn't do this. So my mom asked Grace's mom to share details of what their actual procedure was for decontaminating the work surfaces so we can be consistent with each other.
6. Grace has been leaving the slides to dry after taking off the filter paper. The procedure still says to put the slides right into the test tubes after the filter paper step so I never let it dry first.
7. By the way, our forceps lost a tip because my mom left it in bleach overnight to clean it.

To find out how I can make sure my disinfectants can stay in contact with the glass slide, I did another procedure to test out how long it takes for my cleaners in filter paper to dry up on a glass slide.

1. Cut out fresh pieces of filter paper (2.5cmx4cm) just like the ones we use in the experiment.
2. Soak a piece of filter paper in each of the three disinfectants (Seventh Generation, Method, Natura) for 5 minutes.
3. Put the filter papers on the end of three glass slides (one each) just like in the experiment.
4. Start the timer.

Observations:

7th: It keeps bubbling up under the filter paper. It didn't have very good contact with the slide in the five minutes. I tried to use tweezers to gently tap the raised parts but then different parts would start to bubble up.

Method: It stayed on the slide and did not dry up for over 14 minutes.

Natura: the corners of the filter paper started to curl up before the five minutes were up.

**Analysis:**

I think I'll pour the disinfectants into the jars at least 30 minutes before we have to use them so the bubbles from 7th can settle. The next time, I won't let the filter paper drip so much so they can stay wet longer on the slide. I found out that Natura also makes a larger disinfectant bottle instead of a small spray pen that Grace's mom got for me originally. The small spray did not have instructions to leave on a surface to disinfect, so maybe that's why it dried up fast. Grace's mom said that her disinfectants stayed very moist through the five minutes of contact on the slides. The new Natura bottle is also a spray, but the instruction says it can be left on a surface for 10 minutes to disinfect, so hopefully it should not dry up as easily. I told Grace what I found with the quick test and that I will be using a different version of the Natura. She said she needs to do the test too to see if I am right.

**January 22**

**Experiment #3**

Grace is doing this experiment with my disinfectants this time because she said she wants to know why my results have not been as consistent, but I told her why already.

We put the master broths and control test tubes into the incubator for the third time. I really don't want to have to do this again later.

10:02pm: 32.5 degree C, Control and 2 Master Broths are in the incubator.

**January 23**

2:10pm: took out of incubator

**Observations:**

**Qualitative:**

MS#1 - almost opaque and yellowy

MS#2 - almost opaque and yellowy and with a few floating flakes about 1.5-2mm diameter

Control - clear and yellow

Our moms met to mix the Master Broths together then split them up into one 10ml Master Broth each for me and Grace.

Spectrophotometer results

Broth control 23/01/21:

-0.001

Master Broth:

0.582

**Quantitative:**

|  |  |
| --- | --- |
| Sample | Spectrophotometer Reading |
| Control | -0.001 |
| Master Broth (mixed) | 0.582 |

Times of starting broth

All controls put in at 3:30pm

Inoculated control 1 put in at 4:43pm

Inoculated control 2 put in at 4:58pm

Inoculated control

Tests 1. put in at 4:48pm

Tests 2. put in at 5:03pm

Tests 3. put in at 6:51pm

Took out 1 at 8:45am

Took out 2 at 9:00am

Took out 3 at 10:45am

**January 24**

Clean controls 24/01/21

1. -0.005
2. 0.000
3. -0.001

Inoculated controls 24/01/21

1. 0.651
2. 0.714
3. 0.630

Tests

* 1. -0.001
  2. -0.003
  3. -0.001
  4. -0.003
  5. -0.002
  6. -0.006
  7. -0.002
     1. 0.020
     2. -0.001
     3. -0.004
     4. -0.007

**Quantitative:**

Spectrophotometer Absorbance Readings:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Trial | Clean Control | Control | Test Disinfectant 1 | Test Disinfectant 2 | Test Disinfectant 3 |
| 1 | -0.005 | 0.651 | -0.001 | -0.003 | -0.002 |
| 2 | 0.000 | 0.714 | -0.003 | -0.002 | -0.001 |
| 3 | -0.001 | 0.630 | -0.001 | -0.006 | -0.007 |

**Qualitative:**

|  |  |
| --- | --- |
| Type of tube | Observations |
| General | Yellow color, a little bit of condensation on the side of each tube |
| Clean control | The clean controls were all very clear, can see through the tubes to the other side, smelled a little bit musty |
| Inoculated control | The inoculated controls were all very muddy looking and very smelly, cloudy, king of really light pastel yellow |
| Test Experiment 1 | All the tubes were very clear, actually looked slightly clearer than the control, not much smell |
| Test Experiment 2 | All the tubes were very clear, actually looked slightly clearer than the control, not much smell |
| Test Experiment 3 | All the tubes were very clear, actually looked slightly clearer than the control, not much smell |

**Qualitative Results**

There is not anything that stands out too much. The clean controls were all very clear, the inoculated controls were all very muddy looking, and the tests all actually looked slightly clearer than the control. The controls were yellow-tinted, and the inoculated controls were cloudy and kind of really light pastel yellow.

Analysis:

1. I think we did a much better job cleaning the work surfaces this time so the results showed no contamination. At least Grace and I did the exact same procedure this time to cut down the number of possible factors that can make the experiment go wrong.
2. Still have to find out what is a negative number absorbance.

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**Background Research:**

**Final Report Ideas:**

**January 25, 2021**

Scientific Question:

What is the difference in the amount of bacteria killed by environmentally friendly disinfectants and chemical disinfectants?

Hypothesis:

NOOOOOOOO

**Main Procedure (FOR THE FINAL REPORT!!!)**

**What we can do ahead of time**

1. Make the incubator
2. Set up work station

Day 1:

Materials

1. Falcon test tubes (3)
2. Sterile swabs (3)
3. Tryptic soy broth (15 ml)
4. Sterile saline (1-2ml)
5. Test tube holder with at least 3 slots (1)
6. Permanent marker (1)
7. Barbecue lighter (1)
8. A dirty surface

Procedure

1. Turn on incubator lightbulb to preheat to 32-35 degrees Celsius
2. Sterilize hands
3. Bring materials to work surface
4. Label falcon tubes "Master Broth 1", "Master Broth 2", and "Control"
5. Write date of test on tubes
6. Put falcon tubes into holder
7. Flame lips of falcon tubes to sterilize
8. Flame lip of broth bottle to sterilize
9. Pour 5 ml of broth into each tube and place lid over top to reduce dust contamination
10. Open saline

Control

1. Swirl 1 sterile swab in saline about 6 times
2. Open tube labeled "Control"
3. Swirl swab in tube about 6 times and take out
4. Cap loosely so that if a person lifted the tube by the cap it would not drop but it shakes
5. Write time on tube
6. Put tube into small test tube holder

Master Broths

1. Swirl 1 sterile swab in saline about 6 times
2. Thoroughly swab dirty surface, making sure to cover swab tip
3. Open tube labeled "Master Broth 1"
4. Swirl swab in tube about 6 times and take out
5. Cap loosely so that if a person lifted the tube by the cap it would not drop but it shakes
6. Write time on tube
7. Put tube into small test tube holder
8. Repeat with tube labeled "Master Broth 2"

After Inoculation

1. Bring small test tube holder to incubator
2. Transfer tubes to larger test tube holder
3. Place large test tube holder into incubator
4. Record temperature of incubator after placing test tubes in
5. Make sure the temperature is about 33 degrees celsius, or 32-35 degrees Celsius
6. Wait about 16 hours for bacteria to grow
7. Check temperature multiple times before going to bed to ensure the stability of the temperature

Day 2:

**What we can do ahead of time**

1. Sterilize everything
2. Label test tubes
3. Prep work surface
4. Sterilize slides using oven, 4 hours before experiment
5. Calibrate the Spectrophotometer

**Sterilizing Slides by Baking**

Materials

1. 9\*13 inch baking pan
2. Oven
3. Parchment paper cut to fit bottom of pan
4. 15 new clean glass microscope slides

Procedure

1. Put parchment paper in pan or glass dish
2. Put glass slides in a single layer on top of parchment paper
3. Put pan on middle rack of oven
4. Turn oven to 325 degrees Fahrenheit on convection bake
5. When oven has finished preheating, set timer for 2 hours
6. When timer is up, turn oven off but leave closed for one hour so the glass doesn't crack with the sudden temperature change

**Sterilizing Work Surface and Materials**

Materials

1. 99% isopropyl alcohol (500 ml)
2. 2 aluminum oven liners (17 ¾ inches by 15 ¼ inches)
3. 2% bleach solution wipes
4. Roll of paper towels
5. 3 Mason jars (125ml each)
6. Forceps (1)

Procedure

1. Work Surface
   1. Spray work surface with bleach solution
   2. Wait 5 minutes
   3. Wipe down
   4. Use paper towels soaked in isopropyl alcohol and wipe down bottom of aluminum pans
   5. Put pans on work surface and 4 layers of paper towels on top of each pan
   6. Pour alcohol on top of paper towels and leave to soak
   7. Let sit for 10 minutes
   8. Lift paper towels and drain excess alcohol from pans
   9. Let dry
2. Jars for Disinfectants
   1. Boil enough water in a pot to cover all three mason jars
   2. Lower jars into water
   3. Turn down heat so that there is just enough to keep water boiling for 3-5 minutes
   4. Take out and put on clean surface to dry
3. Sterilizing Forceps
   1. Spray forceps with alcohol and leave to dry
   2. In between putting soaked coffee filters on top of slides, flame with barbeque lighter
   3. Flame in between slides when putting slides into broth
   4. \*WARNING\* DO NOT PUT IN BLEACH, WILL EAT AWAY AT FORCEPS

Using the Spectrophotometer to Measure Turbidity

1. Calibrating the Spectrometer (Spectro Vis attached to the LabQuest2, both from Vernier)
   1. Click on SENSOR menu at the top
   2. Click CALIBRATE
   3. Click USB Spectrometer
   4. Let it warm up
   5. When the meter asks for a blank cuvette, use fresh tryptic soy broth as a BLANK
      1. NOTE: the cuvettes have two opposite clear sides and two ridged sides. Only take the cuvette by the ridged sides because the meter reads the amount of light going through the clear sides
   6. Press "Finish Calibration"
   7. Press "OK"
   8. The red box shows the absorbance reading, which should be very close to 0 for the BLANK
2. Remaining set-up
   1. Select Time-based mode
      1. Tap on Mode box on the right
      2. Press "Time-based" on the drop-down menu
      3. Press "OK"
   2. Change wavelength to 600nm
      1. Tap on the red box at the middle of the screen
      2. Press "Change Wavelength"
      3. Enter "600 in the box
      4. Press "OK"
   3. Double-check calibration
      1. Put the BLANK back into the slot and make sure absorbance is still 0.
      2. Repeat calibration steps if absorbance is not 0
   4. Measure Absorbance of the samples
      1. Put the cuvette with sample in the slot
      2. Record absorbance shown in the red box
      3. Before each set of samples from a different trial, put the BLANK back into the slot to make sure absorbance for the BLANK is still 0

**Experiment setup**

Materials

1. Falcon test tubes (15)
2. Forceps
3. Master Broth (combined tubes into one)
4. Tryptic soy broth (150ml)
5. Test tube holder with at least 15 slots (1)
6. Permanent marker (1)
7. Barbecue lighter (1)
8. Sterilized glass slides (15)
9. Micropipette
10. Micropipette tips (3)
11. Paper templates with 5 diagrams of glass slides on each (3)
12. Plastic protector for each paper template (3)
13. Small scissors (1)
14. Unscented hand sanitizer
15. 99% Isopropyl alcohol spray
16. Incubator
17. Notepad and pencil
18. Timer

Procedure

1. After 16 hours of incubation, take out the Control and Master Broths
2. The four tubes of Master Broths will be mixed together and split into 2x10ml, one for each experimenter
3. Measure absorbance of the Control and combined Master Broth
4. Put down aluminum liners on counter beside incubator
5. Label the four spots on each paper template:
   1. Inoculated Control
   2. Disinfectant 1, 2, and 3
6. Put the paper templates inside plastic protectors
7. Spray plastic protectors with isopropyl alcohol on both sides and wipe down
8. Lay templates on top of aluminum liners
9. Label the jars
   1. Disinfectant 1, 2, and 3
10. Assign the numbers to each brand of test disinfectants
11. Take the jars and pour each halfway full with the assigned test disinfectant
12. Close the lids of the jars for about 20 minutes for any foam to settle
13. Remove the cap from tryptic soy broth
14. Flame the rim of the broth bottle
15. Remove the caps of the test tubes
16. Flame the rim of each test tube
17. Pour 10ml of broth into each labeled test tube
    1. Controls 1, 2, and 3
    2. Inoculated controls 1, 2, and 3
    3. Tests # 1.1, 1.2, 1.3, 2.1, 2.2, 2.3, 3.1, 3.2, 3.3
       1. The first number is the Trial #, the second number is the number assigned to the corresponding disinfectant
       2. For example, Test #2.3 will be the second trial, disinfectant number 3
    4. There should be 15 test tubes
    5. Keep labeled test tubes with broth in them in large test tube holder
    6. Keep the tubes capped when not in use for the trial
18. Put one sterilized glass slide into each of the Controls 1, 2, and 3 tubes
19. Put the rest of the sterilized slides on top of templates according to the outline of the template (4 slides per template)
20. With isopropyl alcohol, clean a pair of small scissors
21. Cut coffee filter paper into nine pieces, measuring 2.5 \* 4 cm each
22. Place three filter papers into each of the three jars to soak for at least one minute

To do each trial on one template

1. Dial the micropipette volume to 5.0µl
2. Gently push the micropipette end into a new sterile tip so it's secure
3. Gently shake the Master Broth so it is cloudy throughout
4. Dip the micropipette into the broth and withdraw 5.0µl amount
5. Push the button on the micropipette all the way down to eject the liquid onto the small rectangle inside the slide, shown on the template
6. Use the micropipette tip to distribute the liquid over the small rectangle
7. Repeat Steps 3 to 6 with the other three slides on the template
8. Let the liquid culture on the slide dry for about 4 to 5 minutes
9. Open the caps of the jars of disinfectants
10. Flame the tip of the forceps and let cool for a few seconds
11. Pick up a piece of disinfectant-soaked filter paper from the first jar
12. Gently shake off excess
13. Place the filter paper on top of the bacteria-inoculated part (the small rectangle) of the slide, making sure the short end of the paper is matching the short end of the slide
14. Repeat Steps 10 to 13 for the other two test disinfectants and corresponding slides
15. Set the timer for 5 minutes
16. After 5 minutes, flame the forceps in between, pick up each filter paper and discard
17. Let the slides dry, about 5 to 10 minutes
18. Put each slide into the corresponding labeled test tubes
19. Cap the tubes loosely
20. Repeat Steps 1 to 19 with the other two Trials on the two other templates

Second Incubation

1. Place the test tube holder with all 15 test tubes in the incubator preheated to about 33 degrees celsius (range 32-35 degrees celsius)
2. Monitor temperature for about an hour to make sure it is steady.
3. Incubate for 16 hours total

Day 3:

**What can we do ahead of time**

1. Calibrate the spectrophotometer with fresh tryptic soy broth as a "Blank"
2. Set up the spectrophotometer for reading absorbance at 600nm absorbance

Materials

1. Spectrophotometer connected to Labquest (1)
2. Cuvettes (16) - one for calibrating with fresh broth and 15 samples

Procedure

1. After 16 hours of incubation, take out the test tube holder with the 15 tubes
2. Calibrate the spectrophotometer if not already done
3. Gently shake the first test tube in case bacteria settled on the bottom
4. Pour into a cuvette to about three quarters full, so when you put the cuvette into the slot in the spectrometer, the liquid comes up above the rim
5. Record the absorbance
6. Repeat the Steps 3 to 5 for the rest of the test tubes and cuvettes

It turns out that Grace did the Materials part. I thought since I was doing the Procedures and we didn't assign the materials part at our last meeting, I would include the Materials, but I guess I have to remove them in the final copy.

**February 13**

Grace's mom, my mom and I met with Dr. Garcia-Dias on Facetime to look at how to do Screencastify on the slides presentation. I think I figured it out but I will have to try it a few times to make sure it works. Grace and I have to figure out which parts we will speak on.

**February 15**

Grace and I met on Facetime to divide up the paragraphs in the Research part:

Intro: Grace

Paragraph one: Me

* Why do we need disinfectants and research about good versus bad bacteria. Different areas in the home that we can keep clean for our health

Paragraph two: Me

* What is a disinfectant and what are the rules around selling disinfectants in Canada
* Environment Working Group, EPA

Paragraph three: Grace

* Types of disinfectants and how they work

Paragraph four:

* Part A - Environmental effects (Grace)
* Part B - Human effects (Audrey)

Paragraph five: Grace

* How to apply different types of disinfectants at home
* How often should we clean anyway and is it effective

Paragraph six: Grace

* Difference between killing viruses and bacteria?
* All six active ingredients we tested are approved by the EPA to fight Covid

Paragraph seven: Me

* Experimental design, the how and the why

Paragraph eight: Me

* In Conclusion...

We also split up the rest of the Scientific Method parts to do:

Results (Grace and me)

Analysis (me)

Future Applications (me)

Improvements (Grace)

Conclusion (Grace)