**Water Testing and Treatment Lab**

**Preparing the samples**

You have been divided into teams and given the following materials:

* A tube of lake water
* Three types of agar plates (red EMB, green citrate and yellow LB)
* Two plastic droppers
* Three Dixie cups
* A Sharpie
* A small bottle of bleach

In order to detect bacteria in the lake water, we must put the water onto plates, where bacteria will grow into colonies.

**Different teams are using different tubes of lake water.** Some tubes contain full strength lake water. Some are ½ strength lake water ( ½ lake water and ½ tap water), ¼ strength lake water (¼ lake water and ¾ tap water) , or only tap water

**Why bother using different concentrations of lake water?** When bacteria grow on plates, they form colonies. Sometimes, there are so many bacteria on the plate that you can no longer see the individual, round colonies. Instead the whole plate is covered in one large mass of bacteria. We want to be able to see the individual colonies, so that we can count them and analyze our results. By using different concentrations of lake water, we can figure out which concentration holds an amount of bacteria that will be easy to analyze.

**Why test straight tap water?** Some of the lake water concentrations have been diluted with tap water. As scientists we need to know if the tap water added any bacteria to our samples so we ‘control’ for any bacteria in the tap water by testing that source separately. We would like to think that the tap water contains no bacteria in it but we also know that places that remain damp (like pipes, tubing and faucets) are great places for bacteria to grow so we can’t rely on what we would ‘like’ to be the case, scientists test it.

**Why couldn’t the teacher just practice this over a vacation, figure out what concentration to do and tell us?** Because the types of bacteria in a water sample are effected by the temperature, every time you take a water sample from a lake you are getting a different population of bacteria. Even the amount of time between when the lake sample was collected and when you plate versus when a science class that meets during a different period plates the water can make a difference. Bacteria divide quickly (about every 30min) so the numbers of individual cells in the population can change quickly.

**Why are the plates different colors?** These plates are examples of selective media which means each media provides different growth conditions where some bacteria can grow on them and others cannot. Selective media can give us a lot of information. You will be plating on LB media, EMB media, and citrate media.

**LB** is a nutritionally rich media that allows most bacteria to grow.

**EMB** media selects for a type of bacteria known as gram negative bacteria. *E. coli* can grow on EMB, Salmonella cannot

**Citrate** media only contains one type of sugar – citrate. Therefore, only organisms that can eat citrate and don’t need other types of sugar will be able to grow on this media. *Salmonella typhimurium* can grow on Citrate media, *E. coli* cannot

By using three types of media we will better understand what type of bacteria are in our sample.**Part 1: Detecting Bacteria in Lake Water**

First, we will plate lake water on the three types of media in order to determine how much, and what types, or bacteria are in our sample.

1. Decide on a name for your team. Using your sharpie, write this name on the sides of all three of the plates you’ve been given.
2. Label the yellow plate “LB”
3. Label the green plate “citrate”
4. Label the purple plate “EMB”
5. Get the dropper that has **no** tape on it. Remove the lid from your LB plate, and use the dropper to put 20 drops of lake water onto your LB plate. Tip and swirl the plate to help the liquid spread evenly across the surface of the agar media. Put the lid back on the plate.
6. Remove the lid from your citrate plate. Use the dropper to put 20 drops of lake water onto this plate, tipping and swirl the plate to help the liquid spread evenly across its surface. Put the lid back on the plate.
7. Repeat the steps again plating 20 drops of lake water onto your EMB plate
8. Make sure all your plates have their lids.
9. Put a small piece of tape on each side of the plate to hold the lid to the bottom.

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| --- | --- |
| **TEAM NAME** |  |
| **TEAM MEMBERS NAMES** | |  | | --- | |  | |  | |  | |  | |
| **WHAT DILUTION OF LAKE WATER WERE YOU TESTING?** |  |

**Part 2: Treating Your Water with Bleach**

As we learned earlier, using bleach is one way to disinfect water.

**But how much bleach is needed to truly disinfect?** In order to test this, we will treat lake water with different amounts of bleach. The final concentrations of bleach in our lake water samples will be 1:5, 1:10 and 1:20. (Note that the team that tested straight lake water in Part 1 will provide the data about how many and what kind of bacteria grow when there is no bleach added)

You have been given three new plates. This time, all *your* plates are the same type of media (all the same color). *Different teams* will be working with a different type of media.

You have also been given a new tube of lake water, and three cups. This tube of lake water is “full strength” lake water.

1. Label the new plates you’ve been given with your TEAM NAME
2. Label one plate “Bleach 1/5,” one “Bleach 1/10” and one “Bleach 1/20”
3. Label the three cups the same way you labeled your plates; one with “Bleach 1/5,” one with “Bleach 1/10” and the other with “Bleach 1/20”
4. *Using the dropper with no tape* on it, put 16 drops of lake water into the “Bleach 1/5” cup.
5. Using the dropper labeled with red tape, put 4 drops of bleach into this cup
6. *Using the dropper with no tape* on it, put 18 drops of lake water into the “Bleach 1/10” cup.
7. Using the dropper labeled with red tape put 2 drops of bleach into this cup.
8. *Using the dropper with no tape* on it, put 19 drops of lake water into the “Bleach 1/20” cup. Using the dropper labeled with red tape, put 1 drop of bleach into this cup.
9. Swirl the water in all three cups to make sure the contents are thoroughly mixed.
10. Using the dropper with red tape, suck all the liquid from the “Bleach 1/5” cup and put it onto the “Bleach 1/5” plate. As before, hold the plate in your hand and swirl it to help the liquid spread evenly across the plate. Put the lid back on the plate.
11. Repeat step 8 using the “Bleach 1/10” cup contents onto the “Bleach 1/10” plate.
12. Repeat step 8 using the “Bleach 1/20” cup contents onto the “Bleach 1/20” plate.
13. Make sure all your plates have their lids on.
14. Put a small piece of tape on each side of the plate to hold the lid to the bottom.

**When you are done preparing your plates**

1. Carefully move your plates to the designated storage area.

2. Clean up your work area remembering that you were just plating bacteria and that bleach is a strong chemical. Make sure stock materials, droppers and waste are taken care of properly.

3. Complete the questions below and be ready to discuss your teams responses with the class. These are *predictions* so it is important to use explaining words like ‘because’ and ‘since’ to show why you are making the prediction that you decided on.

1. Which type of media do you think will allow the largest number of lake bacteria to grow?
2. Which type of media do you think will allow the fewest number of lake bacteria to grow?
3. What do the different types of media select for?
4. If nothing grows on the citrate plates, what does that say about the population of bacteria in your sample?
5. How does bleach kill bacteria?
6. Do you think all three concentrations of bleach will kill all the bacteria?
7. Which bleach concentration will kill the most bacteria?