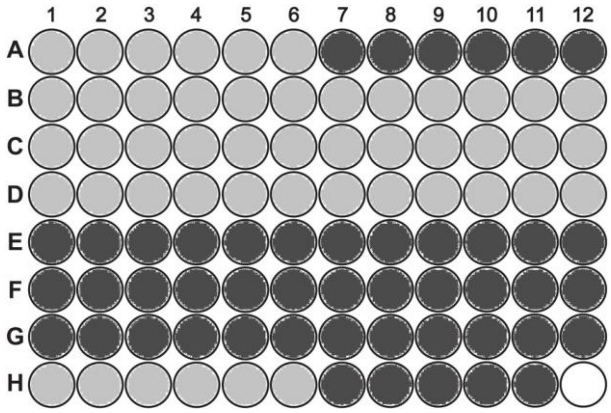
Biofilm Lab

Tips:

- Use mouthwash brands that do not list alcohol as an active ingredient.
- These 4 bacteria species work well: *Escherichia coli, Lactobacillus lactis, Staphylococcus epidermidis, Staphylococcus salivarius*
- Have students fill out a well plate diagram before Day One with exactly what goes in each well.

Day One - Materials:

96uL of bacteria species #1
94uL of bacteria species #2
100uL of water
1.2mL of ethanol
15.4mL of LB broth
9mL of mouthwash
Pipets and tips
Saran wrap
Rubber band
37°C incubator
96 well plate



In this diagram, light gray represents bacteria species #1. Dark gray represents bacteria species #2. White represents the well without bacteria.

Day One: Set up 96 well plate.

1. Row A serves as a positive control. All bacteria in this row should die, and there should be no detection of a biofilm.
    - Put 2uL of bacteria and 100uL of ethanol in each well of Row A. A1-A6 will contain bacteria species #1. A7-12 will contain species #2.

2. Row H serves as a negative control. All bacteria in this row should live, and there should be maximum detection of a biofilm.
    - Put 2uL of bacteria and 100uL of broth in each well of Row H. H1-H6 will contain bacteria species #1. H7-H11 will contain species #2.

3. Well H12 serves as a blank. It will not contain any bacteria, and thus no biofilm. It will be used to adjust the numerical output of the plate reader on Day Three.
    - Put 100uL of water in H12.

4. Rows B-D will be identical so that the experiment with species #1 is run in triplicate.
    - Put 2uL of bacteria species #1, 198uL of broth, and some quantity of mouthwash in each well of Row B. The volume of mouthwash will increase throughout B1-B12 but cannot exceed 125uL of mouthwash since the maximum well volume is 325uL.
    - Repeat for Rows C & D to identically match Row B.

5. Rows E-G will be identical so that the experiment with species #2 is run in triplicate.
    - Put 2uL of bacteria species #2, 198uL of broth, and some quantity of mouthwash in each well of Row E. The volume of mouthwash should match the volumes used in B1-B12.
    - Repeat for Rows F & G to identically match Row E.

6. Use saran wrap and a rubber band to cover plate.

7. Put in 37°C incubator for 2-3 nights.

Day Two - Materials:
3.9 mL crystal violet stain
Pipets and tips, or squeeze pipets
Plastic Tupperware-style bin
Sink

Day Two: Stain biofilm.
1. Dump contents of well plate into sink.

2. Rinse with water. Flick the plate aggressively into sink to ensure all liquid is coming out of wells with each rinse. Continue to rinse and flick at least 5 times.

3. Put 325uL of crystal violet stain into each well. This can be measured precisely, or you can use squeeze pipets and fill each well to the top.

4. Let stain sit for 10 minutes.

5. Rinse and flick repeatedly at least 10 times.

6. Submerge empty well plate underwater in Tupperware for 5 minutes.

7. Repeat step 5.

8. Let well plate dry overnight.

Day Three - Materials:
31.2 mL of ethanol
Pipet & tips
Plate reader

Day Three: Collect data.
1. Put 325uL of ethanol into each well.

2. Put well plate into plate reader.

3. Have students subtract (or calibrate) the numbers that are printed from the plate reader. They will use H12 as a blank, meaning that the readout of H12 identifies the light absorbed by the plastic and ethanol when no biofilm is present. The value of H12 must be subtracted from the value of every single other well so that students are left with numerical data that shows absorbance by the biofilm.

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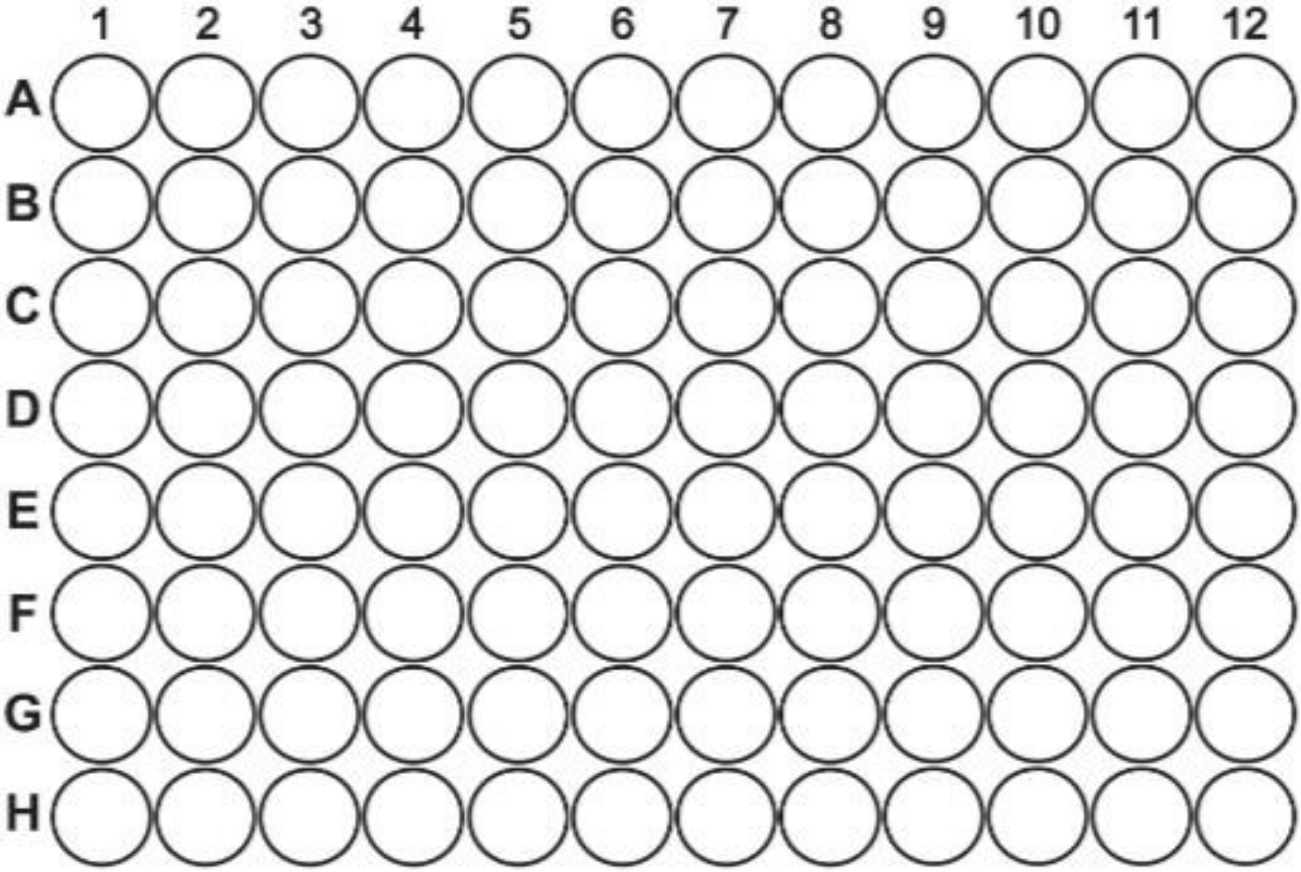

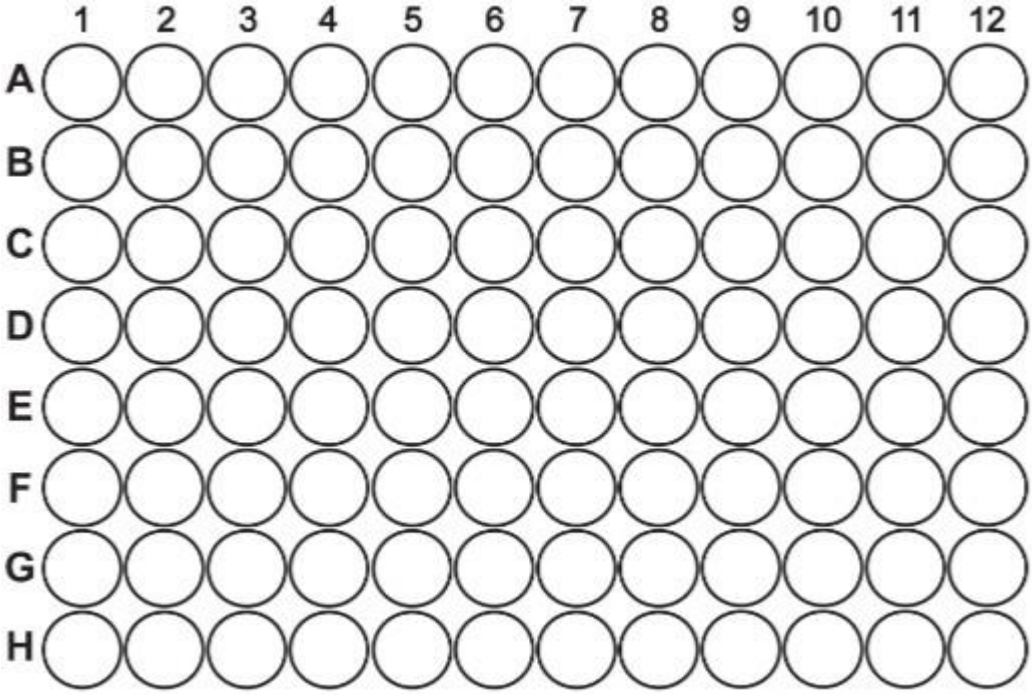

Biofilm Poster Headings and Layout

Create a Google Slide. Your poster must be in the order below:

	Title	
Introduction	Abstract	Results
Methodology	Materials	Discussion

On a 2nd Google Slide, include who in your group was responsible for each part of the poster.

Title

- Include the scientific name of the organism, the independent variable, and the dependent variable.

Introduction

- What question/s are you answering in this lab?
- Give a brief overview of how you answered this question in this activity.
- What is your hypothesis?

Abstract

- What are biofilms?
- Connect this lab with to we learned in class.
- What organism/s are you using? Why?
- What mouthwash are you using? Why?

Methodology

- Write a detailed step-by-step procedure.

Materials

- List all the materials and quantities needed.

Results

- Include any pictures, tables, or graphs that demonstrate your outcome. Include captions for each. **For this lab you must include a computer generated graph of results!
- Explain what the data shows and identify any patterns.

Discussion

- Discuss your results.
- Are your results what you did or did not expect? Explain why/why not.
- What do your results mean to the scientific community and general public?
- Draw a conclusion.
- How would you improve this experiment if you were to do it again?
- What would you explore next to further support your research in this area?