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17 - BACTERIAL RISK ANALYSIS

PURPOSE

To practice running an experimental model and to determine if an artificial sample containing *Escherichia Coli* bacteria would present a health risk for swimmers

MATERIALS

- ☐ *Escherichia coli* in a nutrient broth
- ☐ Petrifilm E.coli/Coliform Count Plates - one plate per group
- ☐ 5 test tubes per group or micropipet strip
- ☐ 1 test tube rack per group
- ☐ a quart of distilled water per class
- ☐ 6 calibrated disposable pipets per lab group or syringes without needles
- ☐ safety goggles
- ☐ 1 beaker or jar with bleach for disinfection per class
- ☐ 25 ml graduated cylinder per each group

PROCEDURE

Use a clean test tube to obtain from your teacher a 1 ml water sample of an *E. Coli* bacterial culture that has already been diluted 1:10 with bottled water. Place the tube in a test tube rack. The lab will be run with a non-pathogenic culture of bacteria to eliminate any real health risks, but we will assume that the sample could be harmful. This artificial sample will represent a sample of surface water that you will test to see if it meets the standards for recreational surface water.

Use sterile technique to add 9 ml of distilled water from a graduated cylinder to the test tube to dilute the original sample. Because the original sample was diluted 1:10, this dilution produces a 1:100 sample. Use another clean test tube and pipet to repeat the process by taking 1 ml of the 1:100 sample and adding it to 9 ml of bottled water for a 1:1,000 (10^4) sample. Continue this serial dilution technique using a clean pipet and test tube each time, until a 1:1,000,000 (10^6) is obtained. Place used pipets in a beaker or jar containing a 10% solution of bleach for disinfection.

Label the final 10^6 dilution as Sample A. Draw up 1 ml into a graduated pipet. Place it in the well of a Petrifilm plate and carefully cover with the top film according to the diagram on the following page. Mark the edge of the paper with an A. Allow the plate to sit undisturbed for 2-3 days.

Then, count the colonies of bacteria growing on the plate. **Do NOT** open the top film - count and observe the growth through the film. Record your data in the chart.

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Examples of how to count colonies:

Figure 1
No growth = 0

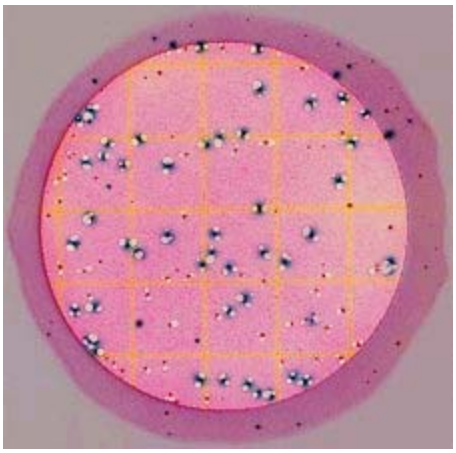

Figure 2
Growth of *E. Coli*

49 (blue colonies with gas bubbles)
Total coliform = 87 (single red and blue colonies with gas bubbles)

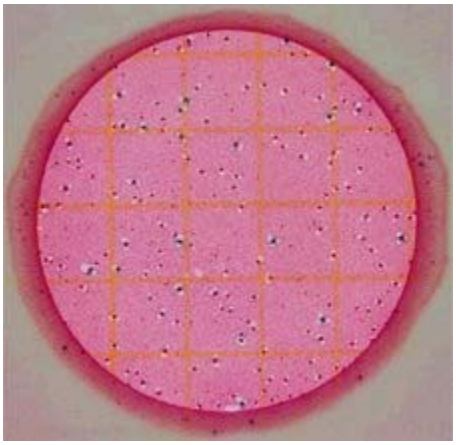

Figure 3

***E. coli* count = 17**
Estimated total coliform count = 150

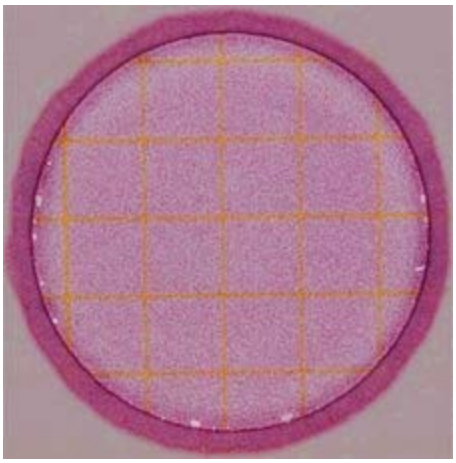

Figure 4

Actual count ~ 10 TNTC (Too Numerous to Count)

Petrifilm EC plates with colonies that are too numerous to count have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel color from red to purple-blue.

Petrifilm E. Coli Count Plates
from 3M Microbiology
Photos used with permission

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DATA

Fill in the data chart with your results:

	Number of E. Coli colonies	Number of Total Coliform Colonies	Sample Dilution Factor (use scientific notation)
Sample A			
Positive Control			
Negative Control			

BACKGROUND INFORMATION

Escherischia coli (*E. coli*) is a bacteria found in the intestines of all warm-blooded animals, including humans. While only a few strains of this bacteria cause disease, their presence in a water sample indicates contamination from sewage or animal wastes. Sewage may contain many kinds of pathogens. The *E. coli* bacteria is used as an indicator species for disease causing agents because it can be easily tested.

Surface water has five classifications:

Class I - potable, drinking water
Class II - shellfish harvesting and mariculture farming
Class III - fishing, wildlife habitats, human swimming and recreation
Class IV - navigation
Class V - industrial discharge

As part of the Clean Water Act, the Environmental Protection Agency requires surface water used for recreation to meet standards (called Class III standards) for coliform bacteria. The standards vary depending on the number of samplings and location. For this lab, we will use the one time sample standard of 800 colonies of fecal coliforms (FCC) per 100 ml of sample. A colony is a single, circular growth when viewed without aid.

Class III Acceptable Risks Table

Dose-response data: @ 800 FCC per 100 ml of sample
8 cases of acute febrile respiratory illness per every 100 swimmers 7 cases of acute gastrointestinal disease with vomiting and diarrhea per every 100 swimmers 6 cases of ear infections per every 100 swimmers

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Assume that your final sample in the petrifilm was not diluted, and was a 1 ml collection. If you multiply the number of colonies by 100, you can determine if the sample would meet water quality standards. (This manipulation of data is because a purchased sample of *E.Coli* is supposed to contain the bacteria and would far exceed the surface water standards.)

Does Sample A meet the standards for Class III water under these conditions? _____

Calculate the expected health risks for your level of FCC count. Set up a ratio like the example.

$$\frac{10 \text{ cases of illness}}{800 \text{ colonies}} = \frac{X = \text{expected number of cases}}{\text{number of colonies identified}}$$

$$\frac{10}{800} = \frac{X}{900} \quad = 11.25$$

Calculate the expected number of cases of illness from the test sample? Record your extrapolations.

	Number of cases
acute febrile respiratory illness	
acute gastrointestinal disease	
ear infections	

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1. What is the question this experiment seeks to answer? _____

_____

_____

2. What is the hypothesis? _____

_____

_____

3. What is the independent variable? _____

_____

_____

4. What is the dependent variable? _____

_____

5. What two factors are correlated in the experimental data? _____

_____

6. What are the limitations of extrapolated data? _____

_____

_____

7. Why are recreational surface waters tested for E. coli bacteria? _____

_____

_____

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8. Why accept any risk? _____

_____

_____

9. What factors cause fecal coliform contamination of surface water? _____

_____

_____

10. What would you do if you knew the bacterial levels were too high for recreational usage in your town, but your surface water (a lake or river) was a popular recreation and tourist destination that produced income for many of the local residents?

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