

LAB EXERCISE: SCIENTIFIC METHOD I

Introduction

The scientific method is the process used by scientists to accurately describe and explain natural phenomena. It consists of several steps. A question is formulated based on observation of natural phenomena, a hypothesis is developed to describe/explain the phenomena, predictions made by the hypothesis are tested by experiment, the data are analyzed, and the hypothesis is accepted or rejected. If the hypothesis is rejected then it is revised based on the additional experimental observations and the revised hypothesis is then re-tested. A hypothesis or a group of related hypotheses that have been confirmed by extensive experimental testing may become a theory or law of nature (*e.g.*, the Germ Theory of Disease). Scientific theories explain natural phenomena and are the basis for development of new scientific knowledge.

In this laboratory exercise the students in the class will learn to use the scientific method to examine a microbiological question. In this lab exercise you will

1. Develop a hypothesis
2. Design an experimental protocol to test your hypothesis
3. Collect data from your experiments
4. Evaluate your hypothesis based on statistical analysis of experimental results
5. Write a lab report

The hypotheses formulated in class will focus on the distribution of coliform bacteria in the environment. Coliforms are a type of bacteria present in large numbers in the digestive systems of animals and are, therefore, associated with feces and fecal contamination. Coliforms are often used as indicators of fecal contamination and the potential presence of human pathogens. For your experiment(s) you will use Path-CHEK swabs for detection of coliforms. These are specific for coliform bacteria with a sensitivity of 13 cfu/cm² of dry surface (environmental *Escherichia coli* on stainless steel). Coliform species detected by the swabs include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp. Instructions for the Path-CHEK swabs can be found at the end of the handout.

STEP 1

Each group of students at a bench will develop a hypothesis to test. The hypothesis should be based on observations and will be tested using the Path-CHEK coliform swabs. For example, based on observations of hand washing, you might hypothesize that men are more likely to have coliforms present on their hands than women. In this lab exercise we will be testing a null hypothesis. A null hypothesis proposes that any variation observed is due to chance and is not statistically significant. The null hypothesis in this example would be: Men and women are equally likely to have coliform bacteria present on their hands. This null hypothesis could easily be tested by swabbing men's and women's hands with the Path-CHEK coliform swabs.

When developing a null hypothesis in class today certain limitations will apply. Limitations to the experiments include: experiments must be performed in the Science Lab Building,

experiments must be performed during the lab period on the day the hypothesis is developed, experiments cannot include direct detection of coliforms on humans, and an area of approximately 10 x 10 cm (or 4 x 4 in) is recommended for swabbing.

Hypothesis developed by lab bench group

STEP 2

The class will select one hypothesis to test from the hypotheses developed today. This is in order to obtain a large enough sample size for statistical analysis of the results.

Class hypothesis to be tested

STEP 3

For a successful experiment the protocol to be followed must be worked out in detail to eliminate variables that might affect the analysis. For example, if you were swabbing hands, should you swab the right or the left hand? Does it make a difference? What if some individuals are right-handed and some are left-handed?

Experimental Design

STEP 4

Data collection. The microbiology lab will be open from 10am until 4pm on Thursday. Check your Path-CHEK tube (see following page) and record the results from your experiment below.

Individual data (describe test performed and result):

Path-CHEK for Coliforms Instructions

1. Each student will perform one test. You will need one pre-moistened Path-CHEK swab (in the large tube) and one Path-CHEK tube containing coliform detection broth (small tube containing purple liquid)¹.
2. Remove the swab from the package and swab as described in your experimental design. (Note: The swab is sterile. Do not contaminate it by touching anything other than the object that you are supposed to swab.) If you need to transport the swab back into the lab, place the swab back into the sterile large tube.
3. Remove the cap from the small tube containing the purple broth and submerge your swab in the broth. While pushing down on the swab bend it to the right and left until the shaft of the swab breaks. Screw the cap back onto the tube (pushing down on the shaft if it extends above the rim of the tube). The swab is now inside the small tube and immersed in the broth.
4. Label the small tube and place the tube into the test tube rack. Tubes will be incubated at 35°C.
5. On Thursday return to the Micro lab, locate your tube, and record the color of the broth (your results). The lab will be open from 10am to 4pm. (Do not try to check your tubes before 10am. There is another class in the lab. Do not disrupt the class!)
 - a. Compare your tube to the negative control (no coliforms present on the swab) and the positive control (inoculated with the coliform, *E. coli*)
 - b. If the media is purple (no color change) your sample did not contain coliform bacteria. Your result is negative. If the media is yellow your sample is positive for coliform bacteria.
 - i. Note: Your tube may not appear identical to the positive control. The intensity of the yellow color may vary. Any yellowish color (or even gray, purple + yellow = gray) is considered a positive result. You may invert the tube to mix the contents. **BUT BE SURE TO CHECK THAT THE TUBE IS SEALED TIGHTLY BEFOREHAND!**
6. Place your tube back into the rack for appropriate disposal later.

DO NOT OPEN YOUR TUBES AFTER INCUBATION FOR ANY REASON!!!

¹ Path-Chek™ kits are used in the food industry to monitor work surfaces and equipment for contamination by potential pathogens. The coliform medium contains selective agents to inhibit the growth of noncoliform bacteria normally associated with different food types whilst allowing uncompetitive growth of any coliform bacteria. The medium also contains a substrate that is metabolised specifically by coliform bacteria. Once the chemical is metabolized the characteristic colour change will occur (*i.e.* purple to yellow).

LAB EXERCISE: SCIENTIFIC METHOD II

STEP 4

Data collection.

Class data

	Positive for coliforms	Negative for coliforms

Notes:

STEP 5

Data analysis.²

You will use a statistical test to decide whether or not to reject your null hypothesis. This test will compare your observed data with the prediction of your null hypothesis. You will determine the probability (P -value) that the deviation between your observed results and the expected results (based on your null hypothesis) is due to chance. The P -value is the probability of getting your observed result if your null hypothesis is actually true. Biologists usually use a P of 0.05 (one chance in twenty) as their breakpoint. If P is less than 0.05 then the null hypothesis is rejected. If P is greater than or equal to 0.05 then you accept your null hypothesis.

The test that you will use to determine if your data supports your null hypothesis is called Fisher's exact test of independence. A chi square statistic is calculated using the observed and expected frequencies and a chi square distribution table is used to determine P . Based on the value of P you will determine if your null hypothesis is supported by the data or if you should reject your null hypothesis. You will need to complete the two tables on page 3 as shown below.

Table X. Observed frequencies (actual data)

	Positive for coliforms	Negative for coliforms	Total
Category 1	(a)	(b)	a + b
Category 2	(c)	(d)	c + d
Total	a + c	b + d	a + b + c + d

Table Y. Expected frequencies (must be calculated)

	Positive for coliforms	Negative for coliforms
Category 1	$\frac{(a + b)(a + c)}{a + b + c + d}$	$\frac{(a + b)(b + d)}{a + b + c + d}$
Category 2	$\frac{(c + d)(a + c)}{a + b + c + d}$	$\frac{(c + d)(b + d)}{a + b + c + d}$

² Handbook of Biological Statistics. <http://udel.edu/~mcdonald/statintro.html>

Enter the class data into Table 1 below. Complete the calculations (for totals) as shown in Table X.

Table 1. Observed frequencies (actual data)

	Positive for coliforms	Negative for coliforms	Total
Total			

Calculate the expected frequencies based on the numbers in Table 1 and the formulas shown in Table Y.

Table 2. Expected frequencies (must be calculated)

	Positive for coliforms	Negative for coliforms

Calculate the chi square statistic for the data set using the equation:

$$\chi^2 = \sum \frac{(\textit{observed} - \textit{expected})^2}{\textit{expected}}$$

(Note: In other words, for your chi square calculation use the observed and expected frequencies for each of the four possible outcomes [category 1 positive, category 1 negative, category 2 positive, and category 2 negative] and add the 4 chi square values together)

Use the chi square distribution table on the next page to determine if the calculated chi square value for the data set exceeds the chi square value required for significance at the 0.05 level ($P < 0.05$). The degrees of freedom for this data set equal one ($df = 1$). Degrees of freedom are

calculated by the number of columns in the table minus one times the number of rows in the table minus one: $(2-1) \times (2-1) = 1$.

Table 3. Chi Square distribution table

Degrees of freedom	Probability (<i>P</i>)					
	0.5	0.10	0.05	0.02	0.01	0.001
1	0.455	2.706	3.841	5.412	6.635	10.827
2	1.386	4.605	5.991	7.824	9.210	13.815
3	2.366	6.251	7.815	9.837	11.345	16.268
4	3.357	7.779	9.488	11.668	13.277	18.465
5	4.351	9.236	11.070	13.388	15.086	20.517

INSTRUCTIONS FOR WRITTEN REPORT

Use the scientific method report template on Blackboard to write your report. In the last section of the report present and discuss your conclusions including, based on your statistical analysis, whether the class hypothesis should be accepted or rejected. Also discuss any problems or errors associated with the experiments including how these errors might affect your results, affect your interpretation of the results, etc. The lab report must be typed with correct spelling and punctuation and **stapled**. The report is due at the beginning of the next lab class.