Laboratory 1
The Scientific Method

The student dedicated to the idea that scientists in general (and perhaps biologists in particular) can help make the world a better place should remember this admonishment: Question everything.

Too often in their daily lives, people read information, assume is correct, and take for granted that those who report the information have gathered it in a logical, rigorous fashion. Unfortunately, this is sometimes not the case. How can the well-informed person know when reported information is flawed? One must consider how the data were collected, whether the sample size was large enough to be representative of reality, and whether the described experiments are repeatable and verifiable.

Critical thinking is a double-edged sword. The scientist who subjects his/her colleagues' work to rigorous analysis (a wise course) must also be willing to submit to such scrutiny from his/her colleagues. In today's lab, you will perform an exercise that will help develop your critical thinking skills and ability to devise good, repeatable experimental design. At the end of the exercise, you will have the opportunity to critique the work of other teams in your lab, and to have your own experimental design analyzed by your classmates. We hope the lessons you learn today will carry you through the rest of your scientific career.

Before you come to lab, it is ESSENTIAL that you read Appendix I in this lab manual. It contains important information about the **Scientific Method**, a set of procedures used by many scientists as guidelines for designing experiments and field studies. There is probably no one scientific method used universally by all scientists, but we present here a general set of guidelines based upon the thoughts of Austrian philosopher Sir Karl Raimund Popper, one of the most influential intellectuals of the early 20^{th} century. Popper insisted that scientific hypotheses be stated in such a manner as to be testable and falsifiable with carefully designed, repeatable experiments.

The scientific method most commonly cited requires that once an investigator has made an observation and wishes to ask a question about some natural phenomenon, s/he must present his/her question the form of a **null hypothesis (H_O).** The unstated hypothesis of interest, the **alternate hypothesis (H_A)**, states the opposite of the null. In short, the Popperian Scientific Method may be summarized as follows. The scientist

1. Observes some natural phenomenon that brings to mind a problem/question
2. Formulates an hypothesis about that problem
3. Predicts whether data to be collected (via controlled experiments or field observations) will reliably indicate whether the null hypothesis is true or false.
4. Designs and executes experiments
5. Analyzes experimental data
6. Rejects (or fails to reject) the null hypothesis, according to data analysis
7. Draws a conclusion about the original question

I. Case Study: Using your Condom Sense.

Below, you will find a **case study** (this can be considered the "natural phenomenon" of step 1 of the scientific method, above), a series of paragraphs outlining a scenario of scientific interest. Work in groups of four to analyze this case study and do the following.

1. Pose questions about the subject of the case study.
2. Choose one of those questions and construct null and alternate hypotheses that will help you answer that question.
3. Make a prediction about results you would expect, were you to perform an experiment to test your hypothesis.
4. Design and perform a careful, rigorous experiment to test your hypothesis.
5. Analyze the data from your experiment.
6. Accept or reject your null hypothesis.
7. Draw conclusions about your original question

The Case Study

As biology majors, possible future health professionals, and concerned members of species *Homo sapiens*, you undoubtedly are aware of AIDS, Acquired Immunodeficiency Syndrome. The causative agent of this disease is HIV, the Human Immunodeficiency Virus.

HIV is transmitted between humans via contact with virus-infected bodily fluids such as blood, semen and possibly saliva. Unprotected sexual intercourse is known to be a major source of infection.

HIV, approximately 116 nanometers in diameter, cannot pass through a synthetic condom. Hence, the use of a condom during sexual intercourse is believed to be an effective way to prevent transmission of the virus from an infected individual to his or her sex partner. Of course, the condom must be intact. Any compromise to the structural integrity of the condom puts the uninfected partner at risk of contracting this ultimately fatal disease.

In some cases, lubricants are used along with synthetic condoms during sexual intercourse. Condoms are made from non-polar petroleum products, as are a number of lubricants, such as petrolatum, mineral oil and some commercial hand and face creams.

Pertinent facts: As you should already know, all matter is made up of atoms and/or molecules. Molecules may be polar (positively charged at one end, and negatively charged at the other) or non-polar (having no difference in electrical charge from one end to the other). For example, water is polar and lipids are non-polar.

In general, polar liquids have the ability to dissolve polar solids, and non-polar liquids can dissolve non-polar solids. That’s why table salt (a polar molecule) dissolves readily in water, and why naphthalene (the substance from which mothballs are made) dissolves in oil. Objects made from petroleum products generally have a relatively high content of non-polar components.

Questions inspired by the Case Study

In the space below, jot down some pertinent questions about solvents and the structural integrity of condoms elicited by the paragraphs above.

II. Designing your experiment

This is perhaps the most critical aspect of the scientific method. The most meticulous measurements in the world will not be useful if you have not formulated a falsifiable null hypothesis and devised logical, rigorous methods for collecting data.

As a group, discuss the questions you wrote down about the case study. Which of these might be answerable with experimental trials you could actually perform in this laboratory in the next two hours or so? Select the most interesting, testable question and move to the next step.

A. Formulating the Hypothesis

As a group, discuss the question you have selected, and then formulate a null hypothesis about that question. Next, state the alternative hypothesis. (You must select in advance whether your alternate hypothesis will be one-tailed or two-tailed. It is dishonest to change your hypothesis after your experimental results have been analyzed. See Appendix I.)

Null Hypothesis:

Alternative Hypothesis:

Make a prediction about what you expect to see:

___

___

___

B. Selecting the test parameters

Once you have constructed your hypothesis, you must determine what parameters will be appropriate to measure in order to test it. For example, if you have asked a question about whether polar solvents damage condom integrity, you might wish to measure some aspect of the condom's physical characteristics (e.g., its volume capacity before breaking, its permeability to various substances, its stretching capacity before breakage, etc.).

Your parameter should be something that can be measured in two different groups, the **control group**, in which all conditions are held constant, and the **treatment group**, in which all conditions are exactly the same as for the control group with the exception of a *single experimental variable*.

You will also have to decide whether to collect attribute, discrete numerical, or continuous numerical data, as described in Appendix I. For example, would it be more informative to measure some parameter that will give you an indication *of the degree to which condom integrity is affected* by a particular substance, or simply whether integrity is affected or not? You must decide which type of data will make your experiment the most powerful and convincing, once analyzed with an appropriate statistical test. Whatever you choose, note that you must decide *in advance* the type of data to collect and which statistical test you will use to analyze your data.

C. Designing the Experiment

Survey the materials and equipment available in the lab. There should be various types of glassware, syringes, meter sticks, rulers and other tools useful for making various types of measurements. There are also tools that you may use to design a mechanical test of condom integrity. Finally, there is a selection of various substances, polar, non-polar, and combined, that could be used in conjunction with a condom during sexual intercourse. Using the available materials (or others you can obtain quickly--be creative), design an experiment to test your hypothesis.

IMPORTANT: If you have decided to collect parametric (continuous numerical) data, you should use *only two different lubricants*, or one lubricant and a control group (receiving a placebo treatment). If you use more than one type of lubricant, you cannot use the two-sample t-test explained in Appendix I. A different statistical test (e.g., ANOVA) must be used to test whether there is a significant difference among multiple continuous numerical means. Unless you already know how to do this, we strongly suggest that you design your experiment to compare the independent means of two different experimental groups. If you choose to collect attribute data, you may still use the Chi-square test. But apply it with caution, and know how to interpret the results.
(HINT: For an experiment such as this one, comparison of treatment and control means will usually yield the most meaningful results.)

1. List the materials your group will use

2. Briefly describe your experimental set-up and procedure.

3. How many times will you replicate your experiment in each of the groups (treatment and control)? ______________________

4. What parameter will you measure? ______________________

5. What statistical test will you use to analyze your data? ______________________

III. Performing your experiment

Once you have designed your experiment and are confident that it has a good chance of yielding analyzable results, collect the materials necessary from the materials supplied in lab and set up your trials.

A. Collecting Data

Remember to keep your treatment and control group as similar as possible, with the exception of the single experimental variable. This means that the same group members should be assigned the same tasks each time, to eliminate as much human error as possible.

Below is a grid for recording your raw data. We will let you label the columns and rows as you see fit, and of course you may not need the entire grid.

Grid for Data Collection:

B. Analyzing Data

A statistical test is a tool used by scientists to determine whether any variation from an expected result is due simply to random error, or due to some non-random phenomenon that bears further investigation. In next week's lab, you will engage in several exercises designed to teach you how to use statistics to analyze your data. We will do a shortened "preview" of such statistics now.

If your team decided to take non-parametric data, refer to Appendix I for instructions on how to apply the Chi-square test. If your team collected continuous numerical data from a treatment and control group, use the following information to analyze your data.

The Independent sample t-test

The measurements you made on your condoms were taken from separate individual condoms in two different test groups. None of the individual condoms were subjected to *paired* measurements, which would mean that the same condom was used as both a treatment and a control. Since each condom was broken or rendered unusable in your experiment, you could not use them in a paired test.

Therefore, we will use a statistical test designed to show whether there is a significant difference between the means of two **independent** samples, those of your (1) treatment and (2) control groups.

Use the following equation to calculate a t-statistic for your two means:

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - 0}{\sqrt{s_p^2(1/n_1 + 1/n_2)}}$$

...where $\mathbf{x_1}$ and and $\mathbf{x_2}$ are the means of your two groups, $\mathbf{n_1}$ and $\mathbf{n_2}$ are the numbers of condoms you used in each group, and $\mathbf{s_p}^2$ is the **pooled variance**, calculated as:

$$s_p^2 = \frac{(n_1 - 1)\,s_1^2 + (n_2 - 1)\,s_2^2}{n_1 + n_2 - 2}$$

...where $\mathbf{s_1}^2$ is the variance of group 1, $\mathbf{s_2}^2$ is the variance of group 1, $\mathbf{df_1}$ is the degrees of freedom for group 1 **($df_1 = n_1 - 1$)** and $\mathbf{df_2}$ is the degrees of freedom for group 2 **($df_2 = n_1 - 1$)**

The **variance (s^2)** for a mean is calculated as

$$s^2 = \frac{\sum(\bar{x} - x)^2}{n-1}$$

In which $\bar{x}$ is the mean, x is each individual value, and n is the sample size. You will learn more about this in the lab on collecting data and using statistical tests to analyze data. But for now, you can just plug in your numbers.

The **degrees of freedom** for a two-sample t-test with independent means is calculated as the sum of the degrees of freedom of each test group:

$$\mathbf{df = (n_1 - 1) + (n_2 - 1)}$$

What is your t-statistic? ____

What are your degrees of freedom? ____________________________________

What is the P value associated with your t-statistic and degrees of freedom? (See Table 1-1.)

___________ > P > ________

<u>Table 1-1.</u> Table of critical values for the two-sample t-test. The P levels (0.05) indicating rejection of the null hypothesis are shown in bold for both one-tailed and two-tailed hypotheses. (From Pearson and Hartley in *Statistics in Medicine* by T. Colton, 1974. Little, Brown and Co., Inc. publishers.)

2-tail -->	0.10	**0.05**	0.02	0.01	0.001
1-tail -->	**0.05**	0.02	0.01	0.005	0.0005
df					
1	6.314	12.706	31.821	63.657	636.619
2	2.920	4.303	6.965	9.925	31.598
3	2.353	3.182	4.541	5.841	12.941
4	2.132	2.776	3.747	4.604	8.610
5	2.015	2.571	3.365	4.032	6.859
6	1.934	2.447	3.143	3.707	5.959
7	1.895	2.365	2.998	3.499	5.405
8	1.860	2.306	2.896	3.355	5.041
9	1.833	2.262	2.821	3.250	4.781
10	1.812	2.228	2.764	3.169	4.587
11	1.796	2.201	2.718	3.106	4.437
12	1.782	2.179	2.681	3.055	4.318
13	1.771	2.160	2.650	3.012	4.221
14	1.761	2.145	2.624	2.977	4.140
15	1.753	2.131	2.602	2.947	4.073
16	1.746	2.120	2.583	2.921	4.015
17	1.740	2.110	2.567	2.898	3.965
18	1.734	2.101	2.552	2.878	3.922
19	1.729	2.093	2.539	2.861	3.883
20	1.725	2.086	2.528	2.845	3.850
21	1.721	2.080	2.518	2.831	3.819
22	1.717	2.074	2.508	2.819	3.792
23	1.714	2.069	2.500	2.807	3.767
24	1.711	2.064	2.492	2.797	3.745
25	1.708	2.060	2.485	2.787	3.725
26	1.706	2.056	2.479	2.779	3.707
27	1.703	2.052	2.473	2.771	3.690
28	1.701	2.048	2.467	2.763	3.674
29	1.699	2.045	2.462	2.756	3.659
30	1.697	2.042	2.457	2.750	3.646

<u>C. Drawing Conclusions</u>

When all groups have completed their trials, analyzed their data and drawn conclusions about their experiments, your TA will lead a class discussion. Answer the following questions carefully.

Does your P value indicate that your two means are different from one another? _

Do you accept or reject your null hypothesis?___________________________________

What is your group’s conclusion about your experiment and original question you posed about the Case Study?

If you were to try to design this experiment again, improvements would you make?

IV. Peer Persuasion.

Once your experiments are complete, each team will have about five minutes to present its findings to the rest of the class. Your team's “job” is to convince your colleagues (classmates) that your original questions, hypotheses and experimental design and analysis are sound. Your colleagues' “job” is to find the holes in your arguments, if they see them. Your TA will act as moderator and Omnipotent Judge.

Each team will present its question, hypotheses, general experimental procedure, results and conclusions to the entire class. You may either appoint a team representative, or take turns presenting the various parts of the project.

When your team has finished its brief overview, your classmates will have a few minutes to ask questions and evaluate what your team has done. You will have a chance to answer their questions and convince them that your experimental design was sound, and that your conclusions accurately reflect reality--or to accept that changes should be made to your experiment, should it be done again by you or someone else.

Remember that the goal is to use your critical thinking skills to help your colleagues to design better, more powerful experiments to test hypotheses.