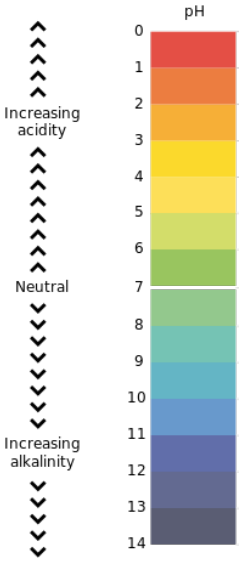
# INVESTIGATION 1: CHEMISTRY OF CELLS

# Pre-Investigation Laboratory #1: *How do we test for organic macromolecules?*

## PART 1: CONCEPT OVERVIEW

**Macromolecules**

Cells are composed of different kinds of organic **macromolecules**, or large molecules: carbohydrates, proteins, lipids and nucleic acids. **Carbohydrates** are molecules that have carbon, hydrogen and oxygen atoms and come in two forms of sugars – **monosaccharides**, meaning "one sugar," and **polysaccharides**, meaning "many sugars. **Proteins** are macromolecules formed from one or more long chains of amino acid residues. **Lipids** are hydrophobic ("water fearing") molecules that are soluble in nonpolar solvents and include triglycerides, waxes, cholesterols and phospholipids. Nucleic acids include molecules such as DNA, RNA, and ATP.




pH (potential of hydrogen) measures the concentration of hydrogen ions and indicates the level of acidity or alkalinity (basic) of a solution. The pH scale ranges from 1-14 with lower numbers indicating more H+ than OH, thus an acid solution, and higher numbers indicating a greater concentration of OH- compared to H+ ions, thus a basic solution.

Scientists can use chemical assays (tests) to determine the molecule or class of molecules found in a substance. In this experiment, you will use different assays to test for the presence of carbohydrates, proteins and lipids, as well as determine the pH of the different substances. A change in the color of the assay will indicate that a certain organic molecule is present.

Image 1: The pH scale.

**GUIDING QUESTION.** *How do we test for organic macromolecules?*

# PART 2: PREDICTION

**Using the table below, describe each of the substances we are using today (i.e. what is Albumin?) and predict which organic molecule(s) they contain**

| Substance | Description | Check all that apply | | | |
|---|---|---|---|---|---|
| | | Monosaccharide | Polysaccharide | Protein | Lipids |
| Water | | | | | |
| Glucose | | | | | |
| Starch | | | | | |
| Albumin | | | | | |
| Broth | | | | | |
| Cream | | | | | |
| Oil | | | | | |
| Coffee | | | | | |

# PART 3: SETUP

**Materials:**

- Benedicts
- Glucose test strips
- Iodine
- Biuret
- Sudan III
- Petri dish
- pH strips
- Ceramic spot plates
- Test tubes
- Water bath
- Substances
- Distilled Water bottle
- p1000 Micropipette
- Pipette tips
- Transfer pipets
- Filter paper

**Getting started:**

- Look at the data table provided. How might you keep track of your substances, i.e. label the glassware in lab?
- What information do you need to record on the data table?
- How can you avoid contaminating each of your substances as you perform the tests?

# PART 4: PROTOCOLS

## Micropipette Techniques

Micropipettes are used to transfer small amount of liquids from one location to another. The volume they pick-up is set manually using a dial with a display below that indicates the volume setting.

The plunger has three positions:

- Rest -the plunger is up and in a "neutral" position
- Stop 1 – the plunger is depressed to the first stop position, and is prepared to pick up the designated volume of liquid
- Stop 2 - the plunger is depressed beyond the first stop position to the second, all liquid in the tip are expelled

**Steps for micropipette use:**

**Liquid Aspiration**

1. Gently, but firmly push the end of the micropipette into the end of a pipette tip. Do not remove the pipette tips from their container by hand. (Use a new tip for each transfer.)
2. Check the volume setting of the micropipette, adjust if necessary.
3. Depress the plunger to Stop 1, place the pipette tip into the desired liquid keeping the pipette vertical and do not submerge the pipette tip more than necessary.
4. Slowly release the plunger until it reaches the Rest position, then depress the plunger again.
5. Repeat step 4 to pre-wet the pipette tip.
6. Slowly press the plunger until it reaches Stop 1 again, then remove the pipette tip from the liquid, keeping the plunger in position at Stop 1.

**Expelling Liquid**

1. Place the pipette tip in the desired container for expelling the liquid. Angle the pipette slightly (less than 45º) and allow the tip to touch the side of the container*.
2. *Slowly* press the plunger to Stop 1, expelling the contents of the pipette.
3. Press the plunger down to Stop 2, forcing any remaining liquid out of the pipette.
4. Lift the pipette out and away from the sample before releasing the plunger.
5. Remove/dispose of the pipette tip by pressing the ejector button beside/below the plunger.

## Benedict's Test

1. Label one test tube for each substance as a Benedict's test using a wax pencil.
2. Place 1 ml of each substance in a separate labeled test tube.
3. Add 2 ml of Benedict's reagent to each labeled tube.
4. Heat all the test tubes for 2-3 minutes in the boiling bath, provided by your instructor (you may heat multiple test tubes at a time).
5. Record any color change on your data table.

## Iodine Test

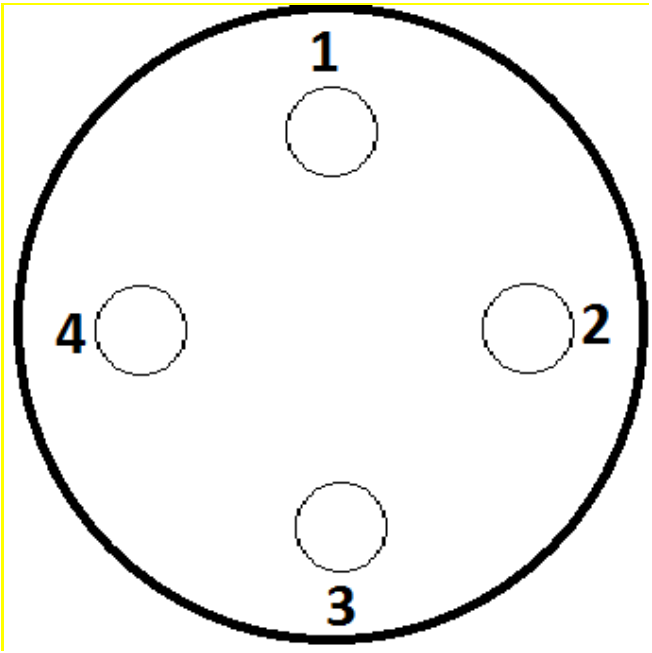
1. Transfer 2 drops of each of your substances in separate wells of a ceramic spot plate.
2. Add one drop of IKI (iodine) solution to each well.
3. Record any color changes in your data table

**Biuret Test**

1. Label one test tube for each substance as a Biuret test using a wax pencil.
2. Place 1 ml of each substance in a separate labeled test tube.
3. Add 3 drops of Biuret reagent to each tube
4. Record any color changes in your data table

**Sudan III**

1. Label two pieces of filter paper with your substances – 4 on each piece (see diagram below). Draw a circle near each number using a pencil.
2. Use a pipet to add a small drop of each substance inside your circle spot on the filter paper.Be certain to avoid contamination of the samples
3. Allow the filter paper to dry. Use a blow dryer if necessary.
4. One at a time, place the filter paper in the jar of Sudan III solution for 3 minutes.
5. Using forceps, remove the filter paper from the dye and rinse for 1 minute in a culture dish with distilled water
6. Record any color changes in your data table




**pH test**

1. Transfer 3 drops of each of your substances in separate wells of a clean ceramic spot plate.
2. Insert a pH paper strip into each well for 30 seconds
3. Allow pH paper to dry and compare the color to the color chart.
4. Record the pH level on your data table.

**Glucose Test**

1. In the spot plate: put 1 ml of each solution in a well of the spot plate.
2. Without touching the pad of the test strip with your fingers, immerse the tip of the strip into the solution for 30 seconds
3. Rub excess substance off the test strip on the side of the well in the spot plate
4. Allow test strip to dry and compare results to indicator scale
5. Record the glucose level on your data table.

**Follow up**

1. Using the data you recorded on your data table, fill in the information on the results table. In the future, you will be creating your own data tables. For this early lab, we have provided you a data table, but you must fill in some of the variables.
2. Using the results table, fill out the conclusions table.

# PART 5: DATA TABLES

**Data table 1:**

| **Substance/Tests** (fill in each test name along the top row) | **pH** | | | | | |
|---|---|---|---|---|---|---|
| **1. Water** | | | | | | |
| **2. Glucose** | | | | | | |
| **3. Starch** | | | | | | |
| **4. Albumin** | | | | | | |
| **5. Broth** | | | | | | |
| **6. Cream** | | | | | | |
| **7. Oil** | | | | | | |
| **8. Coffee** | | | | | | |

## Data table 2: Results

| **Test** (the first row has been started for you) | **Molecules tested for** | **Color of positive** | **Color of negative** |
|---|---|---|---|
| **pH** | | (Base) | (Acid) |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

## Data table 3: Conclusions

| **Substance** | **Check all that apply** | | | | |
|---|---|---|---|---|---|
| | **Monosaccharide** | **Polysaccharide** | **Protein** | **Lipids** | **Acid or Base?** |
| Water | | | | | |
| Glucose | | | | | |
| Starch | | | | | |
| Albumin | | | | | |
| Broth | | | | | |
| Cream | | | | | |
| Oil | | | | | |
| Coffee | | | | | |

# Investigation #1: *What are the three unknown tissues?*

## PART 1: CONCEPT OVERVIEW

**Introduction:** A coworker doing an experiment on plant and animal tissues has mixed up three vials of tissue. Your coworker explains that she had harvested plant root tissue, plant leaf tissue, and animal tissue a couple weeks ago, but had gotten distracted and didn't write down which tissue is in which vial. Now she needs your help to figure out which tissue is which.

**Your task:** You have three different tissues. You must determine the molecular makeup of these tissues in order to narrow down where these tissues come from using the assays provided.

## PART 2: SETUP

**Guiding question:** *What are the three unknown tissues?*

**Materials:**

- Distilled Water bottle
- Benedicts
- Iodine
- Biuret
- Sudan III
- Petri dish
- Filter paper
- Transfer pipets
- Glass pipette
- Pipet pumps
- p1000 Micropipette
- Pipette tips
- Test tubes
- pH strips
- Glucose test strips
- Results table from pre-lab
- Three unknown tissues

**Getting Started:**

- Which macromolecules are present in each of the unknowns?
- Which tissue types have these types of macromolecules?

# PART 3: INVESTIGATION PROPOSAL

**The Guiding Question…**

↓

**What data will you collect?**

↓

**How will you collect your data?**

What safety precaution will you follow?

↓

**How will you analyze your data?**

I approve of this investigation. _____ _____

Instructor's Signature Date

**Data Tables and Observations**

| Substance | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Unknown A** | | | | | | | |
| **Unknown B** | | | | | | | |
| **Unknown C** | | | | | | | |

↓

**Data Analysis and Results Tables**

↓

**Claim**

# Lab Report Investigation #1: *What are the three unknown tissues?*

**Lab Report:** Once you have completed your work, you will prepare an *investigation report* that consists of three sections. Your report should answer these questions in 2 pages. This report must be typed and any diagrams, figures, or tables should be embedded into the document. Generally, you need one page for the first two sections and the second page for third section.

**Section 1:** What concept were you investigating and how does it relate to the guiding question?

- Specifically why is it important to understand which macromolecules are present in different cells and tissues? (See the introduction on chemistry of cells to get started.)
- What is your hypothesis and why did you decide on that hypothesis?

**Section 2:** How did you go about your work and why? This is NOT the details of your procedure, but a discussion and justification of the processes.

- Briefly describe the methods used for measuring different macromolecules.
- How did you measure your dependent variables?

**Section 3:** What is your argument? This third section is where you not only present your data, but also use the values you obtain as evidence in your reasoning. Statements like, "see data table for values" are NOT acceptable.

- State your claim.
- Use the chemistry of macromolecules and how they react to each reagent to support your claim.
- How would you know each macromolecule was present?
- Can you figure out the concentration of the macromolecules or just presence/absence? How do you know?
- Justify your claim.
- Include a data table and refer to the data to support your claim.
- Discuss the validity and reliability of your data.
- Compare and contrast with the other groups results. Discuss reasons for differences.