Title of Lab: # Using Indicators to Test for Compounds

Name_____ Date _____
Period_____

Purpose of the Lab: _____

_____

_____(2 pts)

(How can you determine the presence of a certain organic compound in a substance?)
Hypothesis: _____

_____

_____(2 pts)

Materials:

Indicator solutions as listed below
Unknown solutions T, U, V, W, X, Y and Z
4 test tubes and a test tube rack
Masking tape
Test tube of 95% ethanol (with stopper)
Hot water bath
Test tube holders
Paper towels
Squares cut from brown bags
Goggles!

Indicator Solutions

The presence of certain compounds can be determined through the use of simple tests using indicator solutions. An indicator solution indicates the presence of the compound by changing to a predicted color when the compound it tests for is present. The following is a list of indicator solutions and the substances they test for, as well as the positive result when the compound is present.

Indicator	Compound it tests for	Positive Result indicated by
Benedict's solution	Simple sugars (like glucose)	Blue liquid turns yellow, orange, or red upon heating
Biuret reagent	Protein	Blue liquid turns violet
Indophenol solution	Vitamin C	Purple solution turns colorless
Iodine	Starch	Red liquid turns blue-black

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Silver nitrate	Salt	Clear liquid forms a white precipitate

In addition to these indicator solutions you will be using alcohol, water, and brown paper to test for the presence of lipids. Lipids do not dissolve in water but they are dispersed in 95% ethyl alcohol (ethanol). In addition, lipids that are dripped on a piece of brown bag cause the bag to become translucent (light is visible through the material).

Procedures:

There are 7 tests to complete. Each of the following procedures is a separate test. Each procedure has its own data table. Data table 1 corresponds to test 1, data table 2 goes with test 2, etc… Between each test you will need to wash out the test tubes and re-label them for the next test. **Wear your goggles for the entire lab!**

- 1. Test for Starch
  Obtain 4 test tubes and use the masking tape to label them W, X, Y, and Z. Put a squirt of unknown substance W into the test tube labeled W. Put a squirt of unknown X into the test tube labeled X, etc… **Please do not mix up the droppers!** Droppers that are in substance W should not be used to obtain samples of any other substance. Make a note of the starting color for each substance in Data Table 1. Add 8-10 drops of iodine to each test tube and shake it gently to mix the contents. Note in the data table the color of each solution after adding iodine. Determine whether or not starch is present in each unknown substance. If there is starch present, designate this with a '+' in the appropriate data table column. If no starch is present designate this with a '-' in the table. Clean the test tubes and prepare them for the next test.

- 2. Test for Simple Sugars (Glucose)
  Obtain 4 test tubes and use the masking tape to label them V, W, X, and Z. Put a squirt of the appropriate unknown substance into each labeled test tube. Add 8-10 drops of Benedict's solution to each test tube and shake it gently to mix the contents. Note in data table 2 the color of each solution after adding the Benedict's solution. Heat the test tubes in a water bath for a few minutes until some color change occurs. Record the colors of the solutions after heating them. Determine whether or not sugar is present in each unknown substance and record the results in the data table. Clean the test tubes and prepare them for the next test.

- 3. Test for Proteins
  Obtain 4 test tubes and use the masking tape to label them U, V, W, and Y. Put a squirt of the appropriate unknown substance into each labeled test tube. Make a note of the starting color for each substance in Data Table 3. Add 8-10 drops of Biuret reagent to each test tube and shake it gently to mix the contents. Note in the data table the color of each solution after adding Biuret reagent. Determine whether or not protein is present in each unknown substance and record in the data table. Clean the test tubes and prepare them for the next test.

- 4. Test for Salt
  Obtain 4 test tubes and use the masking tape to label them U, V, Y, and Z. Put a squirt of the appropriate unknown substance into each labeled test tube. Make a note of the starting color for each substance in Data Table 4. Add 8-10 drops of silver nitrate solution to each test tube and shake it gently to mix the contents. Note in the data table the color of each solution after adding silver nitrate. Determine whether or not salt is present in each unknown substance and record in the data table. Clean the test tubes and prepare them for the next test.

- 5. Test for Vitamin C
  Obtain 4 test tubes and use the masking tape to label them U, V, Y, and Z. Put a squirt of the appropriate unknown substance into each labeled test tube. Make a note of the starting color for each substance in Data Table 5. Add 8-10 drops of Indophenol solution to each test tube and shake it gently to mix the contents. Note in the data table the color of each solution after adding Indophenol solution. Determine whether or not Vitamin C is present in each unknown substance and record in the data table. Clean the test tubes and prepare them for the next test.

- 6. Test 1 for Lipids
  Obtain 2 pieces of brown paper bag. Drop a few drops of substance T on one piece and a few drops of substance Z on the second piece. Do not mix the drops on the same piece of brown bag. Note in data table 6 whether or not the substance appears translucent on the paper. Also note whether or not lipids are present.

- 7. Test 2 for Lipids
  Obtain a stoppered test tube containing ethanol and a clean test tube that is half-filled with water. Add a few drops of substance T to each test tube and shake them until the substance is dispersed in each liquid. Wait a few minutes, then record in table 7 whether or not the substance dissolves in each liquid and whether or not lipids are present.

Data and Observations: (24 pts)

Data Table 1: Test for Starch

Test Tube	Substance	Color to Start	Color After	Starch? (+/-)
1				
2				
3				
4				

Data Table 2: Test for Simple Sugars

Test Tube	Substance	Color before heating	Color after heating	Sugars? (+/-)
1				
2				
3				
4				

Data Table 3: Test for Protein

Test Tube	Substance	Color to Start	Color After	Protein? (+/-)
1				
2				
3				
4				

Data Table 4: Test for Salt

Test Tube	Substance	Color to Start	Color After	Salt? (+/-)
1				
2				
3				
4				

Data Table 5: Test for Vitamin C

Test Tube	Substance	Color to Start	Color After	Vitamin C? (+/-)
1				
2				
3				
4				

Data Table 6: Test 1 for Lipids

Brown Bag Piece	Substance	Translucent?	Lipids? (+/-)
1			
2			

Data Table 7: Test 2 for Lipids

Test Tube	Substance	Dissolves?	Lipids? (+/-)
ethanol	T		
water	T		

Analysis Questions:

1) Write the letter(s) of the unknown substances that contain these organic compounds. (7 pts)

Starch _____ Simple Sugars _____

Protein _____ Salt _____

Vitamin C_____ Lipids_____

None of the Above _____

2) Imagine that you take a piece of liver and crush it up, then add water to make a reddish-brown solution. You put some of this solution into 3 test tubes.

Into test tube 1 you add some Biuret reagent.
Into test tube 2 you add some Benedict's solution and heat the tube.
Into test tube 3 you add some Indophenol solution.

You record the following color results:

Test tube 1 = violet
Test tube 2 = yellow-orange
Test tube 3 = bright purple

What organic compounds can you conclude that liver contains? How can you tell? Please answer in complete sentences. (3 pts)

3) Now imagine that you pour some white grape juice into 3 test tubes.

Into test tube 1 you add some iodine.
Into test tube 2 you add some Benedict's solution and heat the tube.
Into test tube 3 you add some Indophenol solution.

You record the following color results:

Test tube 1 = reddish brown
Test tube 2 = orange
Test tube 3 = clear

What organic compounds can you conclude that grape juice contains? How can you tell? Please answer in complete sentences. (3 pts)

4) You drop some of an unknown substance into a test tube that contains water. You shake the test tube up and you notice that the unknown substance is dissolved by the water. Does the unknown substance contain lipids? How can you tell? What other test could you do to be sure? (3 pts)

5) Explain how you would test a solid substance (like a vegetable, or nuts or beans) for the presence of salt? Answer in complete sentences and record your procedure in steps. (3 pts)

6) Starch is made out of long chains of glucose molecules (simple sugars). One source of dietary starch is wheat flour. If you put some flour in water and add iodine it will turn blue-black, indicating that starch is present. If you put some flour in water and add Benedict's solution, then heat it up, it will NOT turn yellow, orange, or red. The glucose chains will not be detected by the Benedict's solution. However, if you put some flour in your mouth, chew on it for awhile, then spit it into the test tube and do the Benedict's test, the solution will change color to yellow-orange. (If your teacher has enough materials and gives you permission to do this you should try it). Why will the Benedict's test show a positive result for glucose after the flour has been chewed on, but not before? Please answer in complete sentences. (3 pts)

Teacher's Page for Indicator Solutions Lab:

This lab, as written, will probably take 2 days, just to get all of the data. Tests can easily be deleted or added depending on time and materials you have available.

The unknown solutions are as follows:

Solution	Contains	Source of
T	Vegetable oil	Lipids
U	Vitamin C powder (dissolved in water…I buy the powder from Trader Joe's)	Vitamin C
V	Knox gelatin dissolved in water (1 pkg per 500 mL works well…must be stirred up often)	Protein
W	Dilute apple juice	Simple Sugars
X	Corn starch suspended in water (about 2 Tbsp/liter of water…must be stirred up often)	Starch
Y	Salt dissolved in water (about 2 Tbsp/liter)	Salt
Z	Distilled water	Control

I usually make up 1 liter of each solution (except the vegetable oil…I only put out about 500 mL of it) for 5 periods of biology. I do not reveal the contents of the unknown solutions to the students (too easy to guess what the positive result should be).

The hardest part about setting up this lab is setting up droppers so that the solutions can be put into test tubes without getting contaminated. I have tried several methods, and the one that seems to work best is to use a 250 mL beaker, labeled with the corresponding letter for the unknown solution, and put 3 or 4 droppers in that beaker. I even label the droppers. I put the 250 mL beakers in front of the appropriate unknown solution, and then I spread the unknowns out as far as possible, to make it difficult to mix up the droppers (I even put them in different places in the room so kids have to walk from station to station to get their unknowns). This actually helps in other ways. The kids split up the chore of getting their unknowns (you go this way, I will go that way) so more people are participating, and there is less crowding around one spot in the room to get the solutions.

The other part of this lab that takes some time to set up is the indicator solutions. Some of these can easily be purchased, but some are easier to mix up on your own. I make about 250 mL of each solution, pour some into 6 dropper bottles, and make 6 baskets of indicator solutions for groups to share. (Each dropper bottle holds about 25 mL so there is usually leftover solution, which you will need when the solutions get low in the bottles). I print out labels for the bottles using Avery address labels (included on the CD-Rom for

this lab) and just stick them on the dropper bottles. When the lab is over I empty the bottles and soak them for a day, then rinse them out and the labels come right off. Here is the table of indicator solutions, how to mix and store them, and whether or not they are hazardous. Always wear goggles when mixing chemicals.

Indicator	To Make	To Store	Warnings
Benedict's Solution	▪ **This is pretty inexpensive and I buy it ready-made.**	▪ **It has a great shelf-life…it lasts for years.**	▪ **It is poisonous so it is considered a moderate health hazard and should be stored in a locked chemical storeroom.**
Biuret Reagent	▪ **Buy it ready-made (it can be hazardous to prepare on your own…it contains sodium hydroxide, a strong base))**	▪ **It will last a few years on the shelf. It starts to get precipitates after awhile.**	▪ **It is poisonous and contains a strong base, so it is likely to sting if it gets on someone's skin. It could be really bad if it gets in the eyes, so make sure goggles are worn. It should definitely be kept in a locked chemical storeroom.**
Indophenol Solution	▪ **Buy some 2,6 dichloroindophenol sodium salt (about 5 grams…it will last a long time).** ▪ **You mix 0.1 gram of this with 100 mL of water to make the solution.** ▪ **It does not store well at all, so only mix up as much as you need.**	▪ **It will last a few weeks in a sealed glass container.**	▪ **This one is corrosive so wear gloves while preparing it. If you have to pour any down the drain make sure it is well-diluted and only pour in small amounts.**
Lugol's Iodine Solution	▪ **This can be purchased ready-made but it doesn't last long, so buying the ingredients and mixing it is a better idea.** ▪ **You dissolve 10 g of potassium iodide powder (KI) in 100 mL of distilled water and** ▪ **add 5 g of iodine crystals.** ▪ **Then you add 500 mL of distilled water.**	▪ **It will last for a few months in a sealed glass container.**	▪ **This is a corrosive liquid so gloves should be worn while preparing. If it gets on skin it should be washed with lots of water and soap.**
Silver Nitrate	▪ **Buy it ready-made as a solution (0.1Molar). If you have the crystals already,** ▪ **prepare a 0.1 M solution by** ▪ **dissolving 1 g of crystals into** ▪ **100 mL of solution.**	▪ **This will last for years on the shelf if kept in a sealed glass container that is tinted (brown) and kept out of the light.**	▪ **This is corrosive and a minor health hazard at this dilution. It should never be poured down the drain. It will cause brown stains on skin and clothing.**

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