Biologically Important Molecules (Macromolecules)

ISHIK UNIVERSITY

BIOLOGY LABORATORY – WEEK 4

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Learning Objectives

By the end of this exercise you should be able to:

1. Perform tests to detect the presence of biologically important carbohydrates, proteins, lipids, and nucleic acids.

2. Explain the importance of a positive and a negative control in biochemical tests.

3. Use biochemical tests to identify an unknown compound.

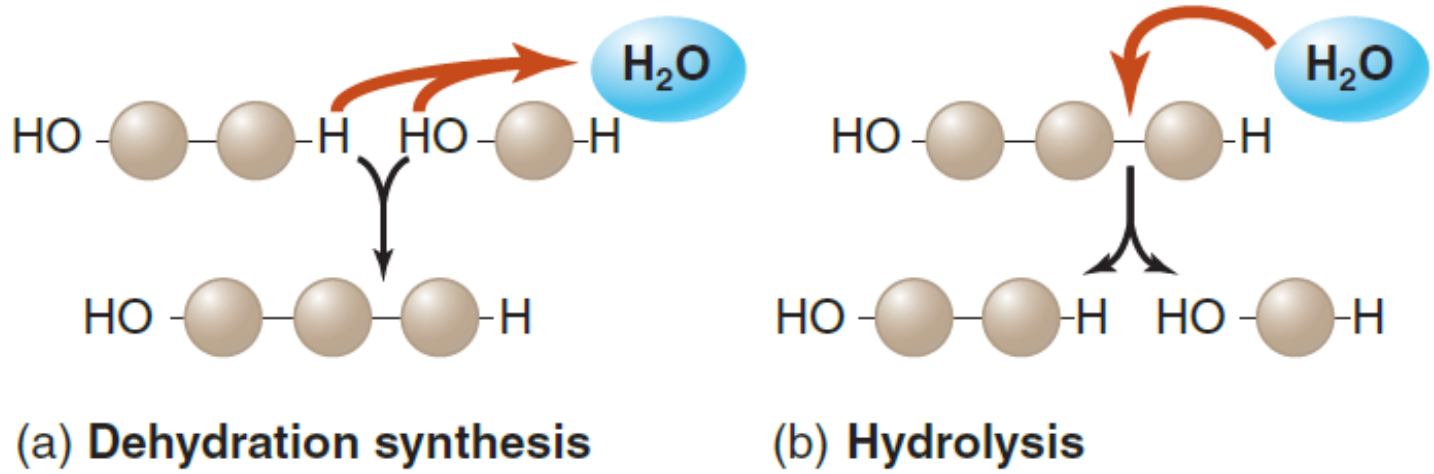
4. Discuss the role of chemistry in the biological sciences.

5. Define compounds and molecules.

Macromolecules

- Most organic compounds in living organisms are: **carbohydrates, proteins, lipids,** or **nucleic acids.**

- Each of these macromolecules is made of smaller subunits. These subunits are linked by **dehydration synthesis,** which is an energy-requiring process in which a molecule of water is removed and the two subunits are bonded covalently.



(a) **Dehydration synthesis** (b) **Hydrolysis**

Macromolecules

- Similarly, breaking the bond between the subunits requires the addition of a water molecule and releases energy. This energy-releasing process is called **hydrolysis.**

- The subunits of macromolecules are held together by **covalent bonds** and have different structures and properties. For example, lipids (made of fatty acids) have many C—H bonds and relatively little oxygen, while proteins (made of amino acids) have amino groups (—NH2) and carboxyl (—COOH) groups.

- These characteristic subunits and groups impart different chemical properties to macromolecules

CONTROLLED EXPERIMENTS TO IDENTIFY ORGANIC COMPOUNDS

- Scientists have devised several biochemical tests to identify the major types of organic compounds in living organisms. Each of these tests involves two or more treatments: (1) an **unknown solution** to be identified, and (2) **controls** to provide standards for comparison.

- A **positive control** contains the variable for which you are testing; it reacts positively and demonstrates the test's ability to detect what you expect.

- A **negative control** does not contain the variable for which you are searching. It contains only the solvent (often distilled water with no solute) and does not react in the test. A negative control shows you what a negative result looks like.

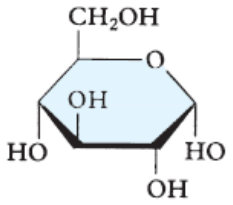
CARBOHYDRATES
Benedict's Test for Reducing Sugars

- Carbohydrates are molecules made of C, H, and O in a ratio of 1:2:1 (e.g., the chemical formula for glucose is $C_6H_{12}O_6$).

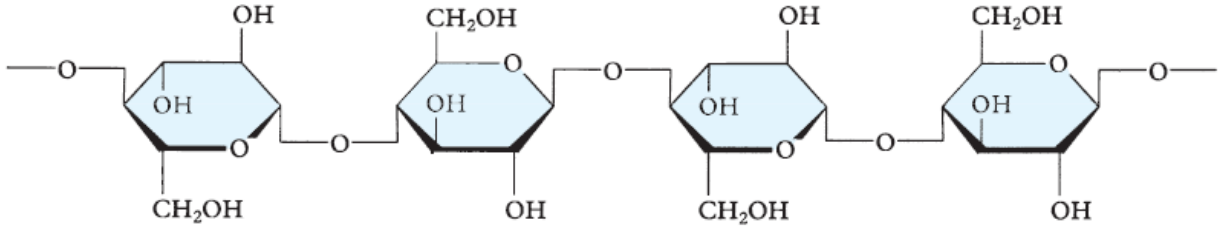
- Carbohydrates are made of **monosaccharides,** or simple sugars.

- Paired monosaccharides form **disaccharides**—for example, sucrose (table sugar) is a disaccharide of glucose linked to fructose.

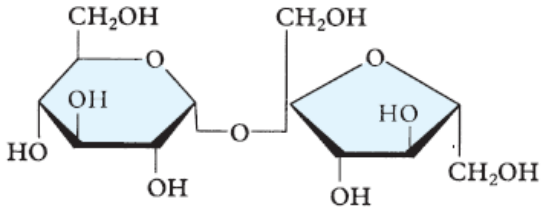
- Similarly, linking three or more monosaccharides forms a **polysaccharide** such as starch, glycogen, or cellulose



Glucose, a monosaccharide



Polysaccharides are polymers of monosaccharides



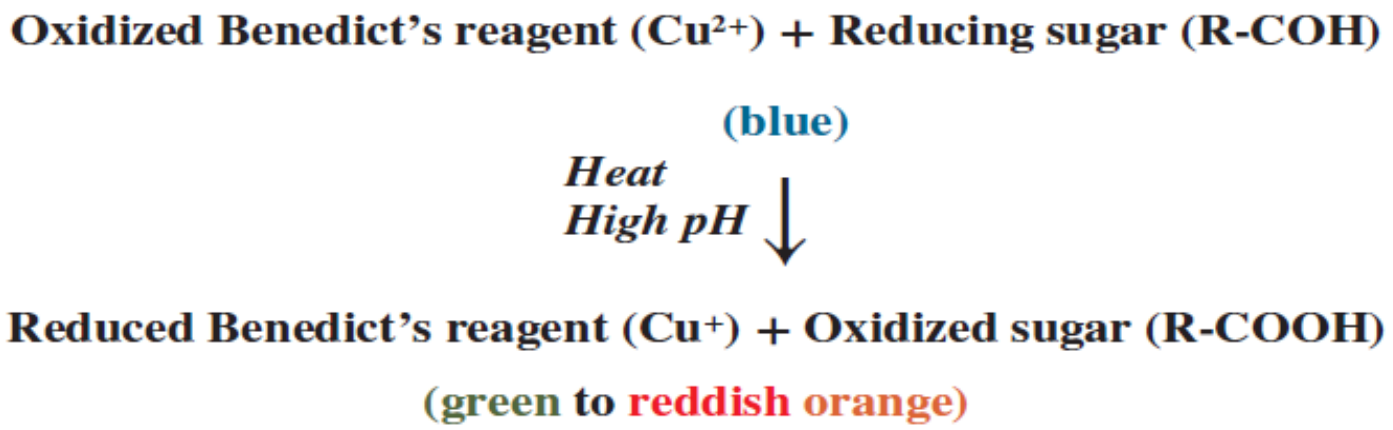
Sucrose, a disaccharide made of glucose (left) and fructose (right)

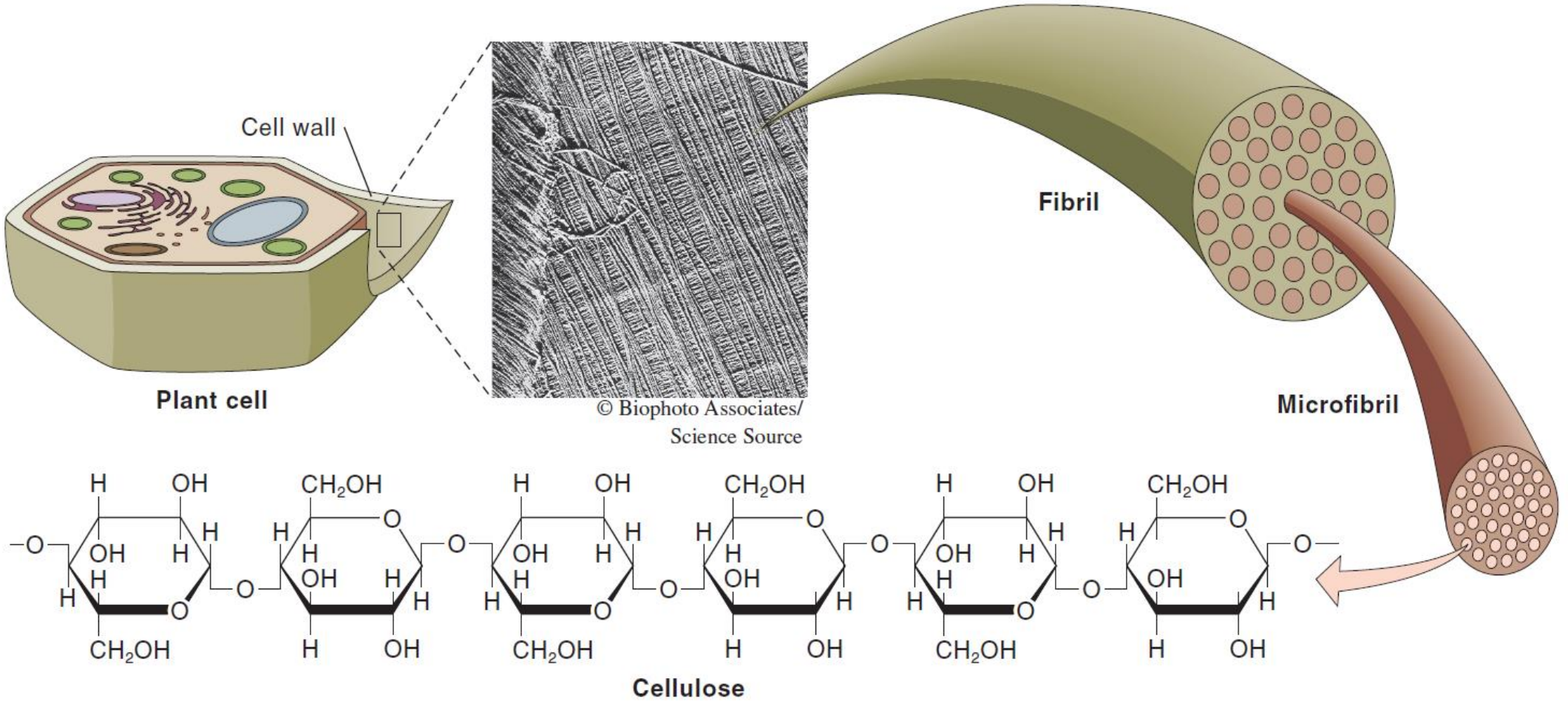
Which groups of a glucose molecule are involved in forming a polysaccharide?

- Many monosaccharides such as glucose and fructose are **reducing sugars,** meaning that they possess free aldehyde (−CHO) or ketone (−C=O) groups that reduce weak oxidizing agents such as the copper in Benedict's reagent.

- **Benedict's reagent** contains cupric (copper) ion complexed with citrate in alkaline solution.

- Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu2+) ions to cuprous (Cu+) oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

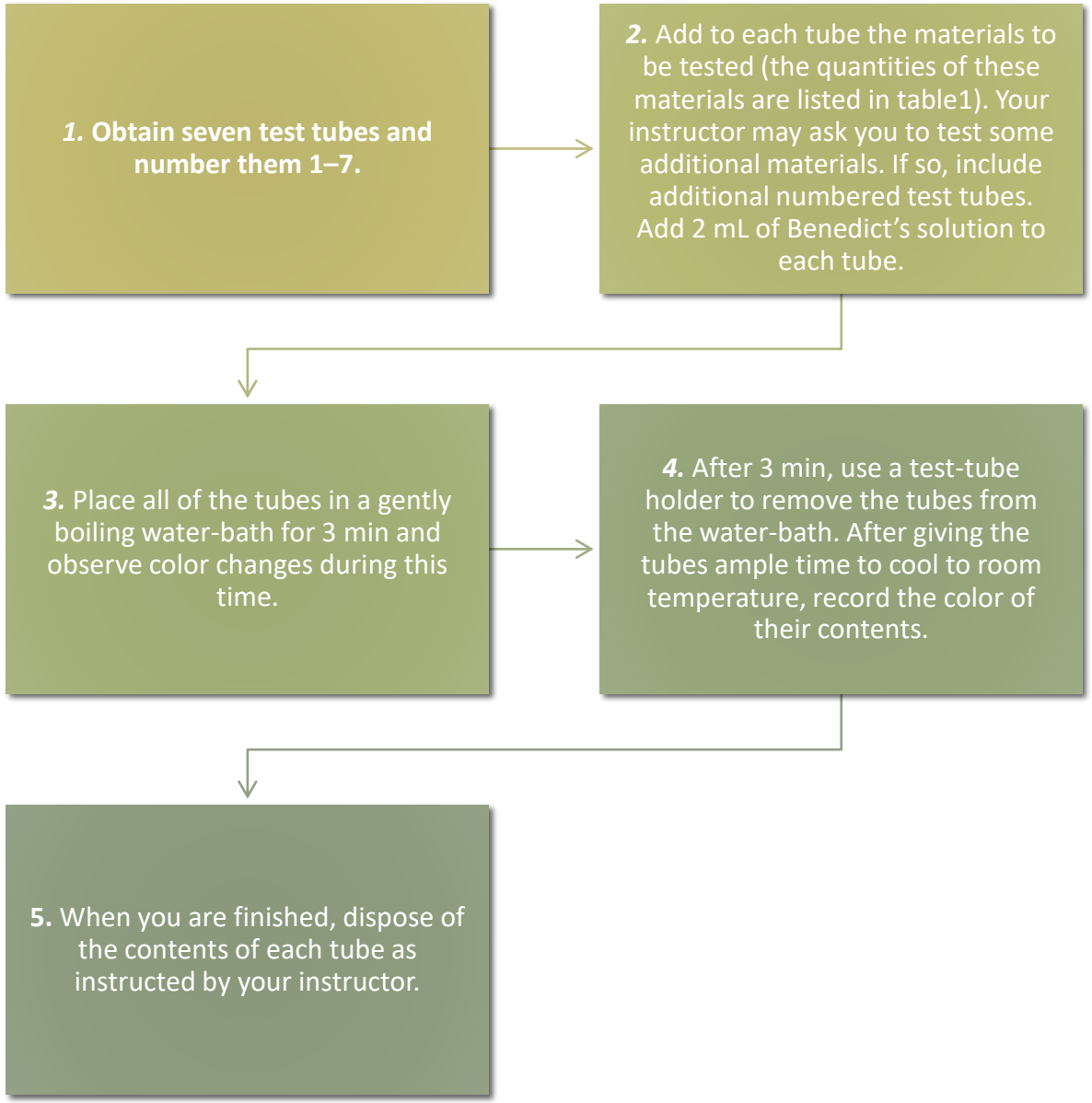



Cell wall
Plant cell
© Biophoto Associates/
Science Source
Fibril
Microfibril
Cellulose

Observation and Results

- A green solution indicates a small amount of reducing sugars, and reddish orange indicates an abundance of reducing sugars. Nonreducing sugars such as sucrose produce no change in color (i.e., the solution remains blue).

Perform the Benedict's test for reducing sugars



Solutions and Color Reactions for (1) Benedict's Test for Reducing Sugars and (2) Iodine Test for Starch			
Tube	**Solution**	**Benedict's Color Reaction**	**Iodine Color Reaction**
1	10 drops onion juice		
2	10 drops potato juice		
3	10 drops sucrose solution		
4	10 drops glucose solution		
5	10 drops distilled water		
6	10 drops reducing-sugar solution		
7	10 drops starch solution		
8			
9			

a. Which of the solutions is a positive control? Negative control?

b. Which contains more reducing sugars, potato juice or onion juice? How do you know?

c. What does this tell you about how sugars are stored in onions and potatoes?

Iodine Test for Starch

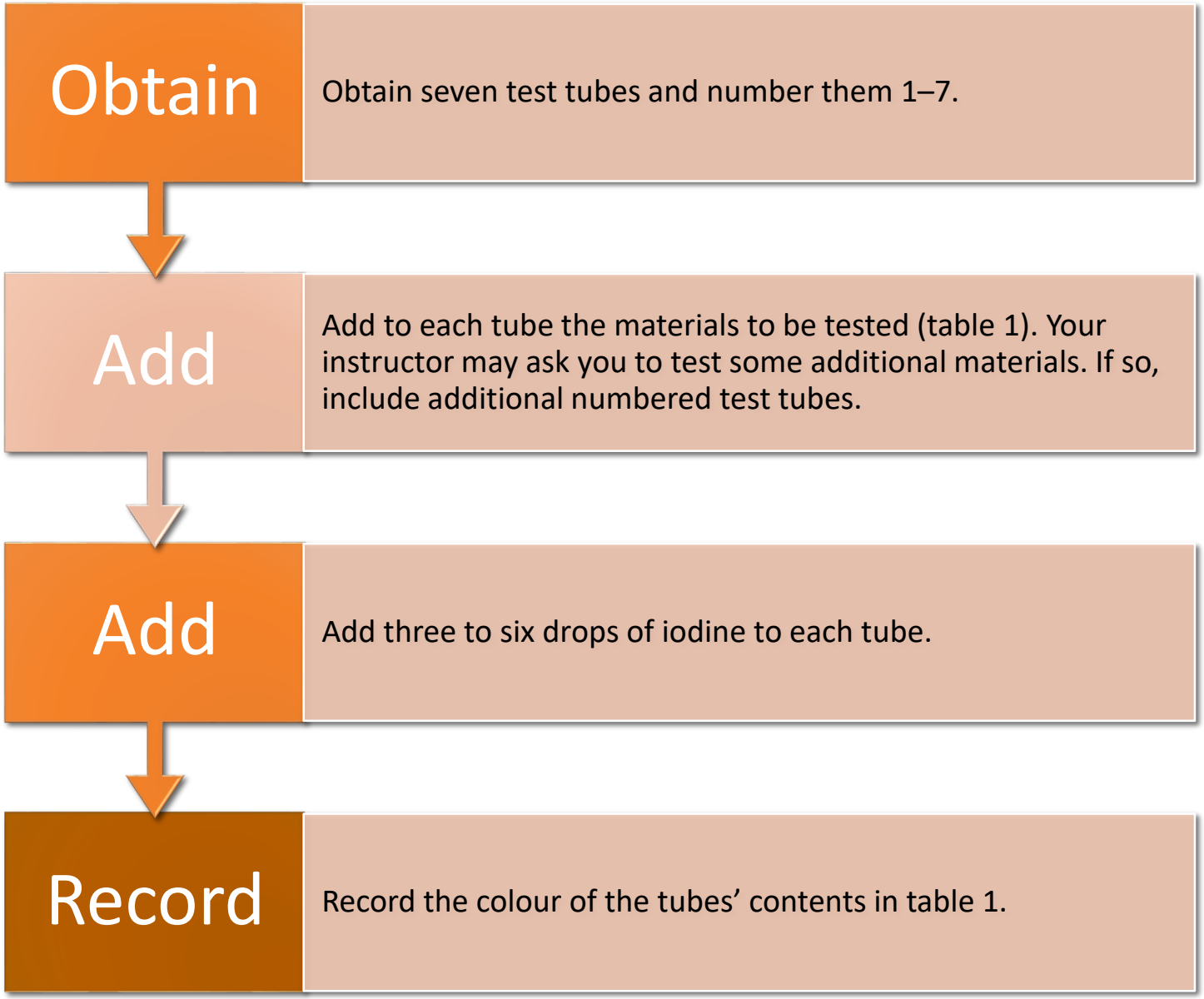
- Staining by iodine (iodine-potassium iodide, I2KI) distinguishes starch from monosaccharides, disaccharides, and other polysaccharides.

- The basis for this test is that starch is a coiled polymer of glucose; iodine interacts with these coiled molecules and becomes bluish black.

- Iodine does not react with carbohydrates that are not coiled and remains yellowish brown. Therefore, a bluish-black colour is a positive test for starch, and a yellowish-brown colour (i.e., no colour change) is a negative test for starch.

- Glycogen, a common polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Perform the iodine test for starch



a. Which of the solutions is a positive control? Which is a negative control?

b. Which colours more intensely, onion juice or potato juice? Why?

c. In what parts of a plant is the most starch typically stored?

d. What are the functions of carbohydrates in living organisms?

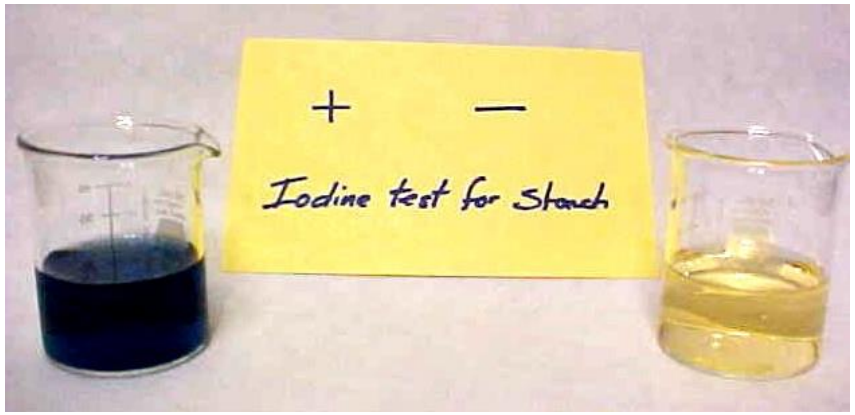
What is an indicator?

Indicators are chemical compounds used to detect the presence of other compounds.

Test for Simple Carbohydrates
Benedict's solution

Aqua blue: negative test.

yellow/green/brick red, etc.: positive test



Test for Complex Carbohydrates Lugol's solution

IKI solution → (Iodine Potassium Iodine) color change = blue to black