

## **Experimental Procedure**

### **A- Molisch's Test**

1. Add 2 ml of 1% solutions of glucose, sucrose and starch into three separate test tubes (i.e each sugar solution into a separate tube).
2. Add 2 drops of Mollsch's reagent into each test tube and mix well by shaking.
3. Incline the tubes so that they point a way from you and from your neighbors. Pour slowly and carefully about 3 ml of concentrated sulfuric acid down the side of the tubes to form a layer below the sugar solution. A reddish-violet ring at the junction of two liquids denotes a positive test. Do not shake the tubes, otherwise the ring disappears and the color distributed all over the solution.
4. Report your observations.

### **B. Anthrone Test:**

1. Add 0.2 ml of 0.1% solutions of glucose, sucrose and 1% starch into three separate test tubes.
2. Carefully add 2 ml of anthrone reagent (i.e 0.2% anthrone in conc.  $M_2SO_4$ ) and mix the tubes thoroughly.
3. Heat the test tubes in a boiling water bath for 3 minutes.
4. Cool the tubes under tap water and observe the color. Record your observations.
- 5- Repeat the test with a few pieces of filter paper and record your observations.

### **C. Bial's orcinol Test:**

1. Add 3 ml of Bial's reagent into three separate test tubes.
2. Add 0.5 ml of 1% solutions of xylose, glucose, and lactose into the test tubes respectively and mix well.
3. Heat the tubes in a boiling water bath until a color develops.

4. Report your observations as to what color developed, and record the time required for the formation of the corresponding color.

#### **D. Seliwanoff's Test:**

1. Add 3 ml of Seliwanoff's (Resorcinol) reagent into each of the four separate test tubes.
2. Add 3 drops of 1% solutions of fructose, glucose, sucrose and starch, one sugar solution per tube. Mix the contents of the tubes.
3. Heat the tubes in a boiling water bath for 10 minutes.
4. Report your observations and accurately record the time required for the color formation in each tube.

#### **II. Effects of concentrated HNO<sub>3</sub> solution:**

##### **Mucic Acid Test:**

1. Place 10 ml of 1% glucose, galactose and lactose solutions into 3 test tubes separately.
2. Add 1 ml of H<sub>2</sub>O and carefully 3 ml of conc. HNO<sub>3</sub> into each tube.
3. Place the tubes in a boiling water bath in a fume hood for 1.5 to 2 hours.
4. Cool the tubes in an ice bath. Scratch carefully the inner walls of the tubes with a glass rod to facilitate crystallization. Record your observations.

##### **Reagents:** How to prepare

1. **Molisch's Reagent:** dissolve 10 g of  $\alpha$ -naphthol in 100 ml of ethyl alcohol 95%.
2. **Anthrone Reagent:** dissolve 2 g of Anthrone in 11 conc. H<sub>2</sub>SO<sub>4</sub>.
3. **Bial's Reagent:** dissolve 300 mg of orcinol in 100 ml of conc. HCL and add 0.25 ml of ferric chloride solution (10 g/100 ml).
4. **Seliwanoff's Reagent:** dissolve 50 mg of resorcinol in 33 ml of conc. HCL and dilute to 100 ml with H<sub>2</sub>O.

