Barfoed's test:

This test is used to distinguish reducing monosaccharides from reducing disaccharides. Since the monosaccharides reduce cupric ions (Cu⁺²) faster than disaccharides even in slightly acidic solution. The rate of reduction depends upon the concentration of cupric ions and the time of heating.

Procedure:

- 1) Add 5 drops of the sugar solution to 15 drops of Barfoed's reagent in a test tube.
- 2) Boil for 3 minutes, and allow to stand. Report your observations.

Seliwanoff's test:

This test is used to distinguish an aldohexose from ketohexose. Heating with HCL dehydrates hexoses to hydroxymethyl furfural (HMF). Ketohexoses yield large amount of HMF and at faster rate than do aldohexoses. HMF form red condensation product with resorcinol.

Procedure:

- 1) To 15 drops of Seliwanoff's reagent add 10 drops of sugar solution (fructose or glucose).
- 2) Place the tube in boiling water bath, record the time needed for your result for each sugar used. A red color develops with fructose (a ketohexose) and no such color with glucose (an aldohexose).

Notes:

- 1) Sucrose also gives a positive test because it is readily hydrolysed during the course of the test yielding fructose as one of the products.
- 2) The time factor in Seliwanoff's test is very important.

Bial's test:

This is specific for pentoses.

Procedure:

1) To 15 drops of Bial's reagent add 5 drops of pentose solution (Xylose or arabinose) and heat in boiling water bath for 3-4 minutes, to get a blue-green color.

Reagents:

- 1) Benedict's reagent: Dissolve 173 gm of sodium citrate, 100 gm sodium carbonate in 800 ml warm water. filter into a 1000 ml measuring cylinder and make up to 850 ml with distilled water. Meanwhile, Dissolve 17.3 gm of copper sulfate in about 100 ml H₂O and make up to 150 ml pour the first solution into a 2 L beaker and slowly add the copper sulphate solution with stirring.
- 2) **Barfoed's reagent:** Consists of 6.5% copper acetate in 1% acetic acid.
- 3) **Bial's reagent:** Is made by dissolving 0.3 g orcinol in 100 ml conc. HCL and then adding 5 drops of 1% solution of ferric chloride.
- 4) <u>Seliwanoff's reagent:</u> Is made by dissolving 0.05 g of resorcinol in 100 ml of dilute (1:2) HCL.