Standard Operating Procedure-I

**Product Name: CRYO-FRUCTO~QUANT (QUANTITATIVE Fructose ESTIMATION KIT)**

Fructose is a marker for seminal vesicle function. Its function in the seminal plasma is as a substrate for the glycoltic (Anaerobic) metabolism of the spermatozoa. This is an important energy source for the sperm and exclusion of the seminal vesicular component from the ejaculate will result in almost completely immotile sperm.

Fructose levels in semen are androgen dependent. Fructose is secreted by seminal vesicles. Fructose levels should be determined in any patient with Azoospermia and especially in those whose ejaculate volume is less than 1 ml, suggesting seminal obstruction or atresia or ejaculatory tract duct obstruction. Absence of fructose, low semen volume, and failure of the semen to coagulate indicate either congenital absence of the vas deference and seminal vesicles or obstruction of the ejaculatory duct.

Disorders of the seminal vesicles and a subsequent reduction in the fructose concentration in semen will also result in a reduced motility of sperm. As the seminal vesicles contribute more than half of the total volume of semen, absence of seminal vesicular secretions will reduce semen volume. Thus the measurement of fructose concentration in men with low volume ejaculates may be of great value diagnostically.

Another situation where fructose estimates are helpful is in men with polyzoospermia and low motility. Occasionally in men with very high sperm concentrations (more than 350 million sperm per ml), the sperm are immotile due to relative deficiency of fructose. If this semen picture is present, the diagnosis can be confirmed by finding very low concentrations or even absence of fructose in semen.

**CRYO-FRUCTO~QUANT Reagents:**

1. Precipitating Reagent, 10 % TCA for precipitation of semen proteins.
2. Color Reagent, Indole-3-acetic acid reagent.

**EQUIPMENT REQUIRED**

1. Test tubes
2. Test Tube Holder
3. Droppers
4. Pipettes
5. Boiling water bath
6. Incubator at 37°C.
7. Colorimeter

**Storage and Stability:**
Store at 2-8°C till expiry date indicated on label.

**CRYO-FRUCTO~QUANT Method:**

1. Mark one tube for calibrator and one tube for each sample. Pipette 0.9 ml precipitating reagent in each tube.
2. Pipette 0.1 ml of calibrator in calibrator tube and 0.1 ml well mixed semen sample in each labeled tube. Mix well and allow 10 minutes for precipitation.
3. Centrifuge all sample tubes at 1500 RPM for 5 minutes to settle the precipitated proteins and obtain clear supernatant.
4. Label another set of tubes and transfer 50 micro-liters of diluted calibrator and sample supernatant to appropriately labeled tubes. Add 50 micro-liters color reagent in all the tubes.
5. Add 2 ml concentrated Hydrochloric acid to each tube and mix well.
6. Incubate at 37°C for 75 minutes. Keep all the tubes well covered with Para film or plastic foil.
7. Read absorbance at 520 nanometer or equivalent filter on colorimeter.
8. Calculate using following formula

\[
\text{Concentration of test} = \frac{O.D_{\text{Test}}}{O.D_{\text{Standard}}} \times 500 \text{ mg/dl}
\]

Assay levels and interpretation:
Concentration of fructose in semen ranges from 63 to 500 mg/dl (3.5 to 28 mmol/l). It must be remembered that as sample ages; the fructose level will fall due to utilization of fructose by spermatozoa. The more sperm that is present in the ejaculate, the greater the fall in fructose concentration.

WARNINGS AND PRECAUTIONS
All human, organic material should be considered potentially hazardous. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens. The reagent contains Hydrochloric Acid which is highly corrosive and irritant. Proper safety gear should be used while performing the test. The reagent should be properly disposed.