

# FRUCTOSE IN SEMINAL FLUID

## UV method for determination of D-fructose in semen

### TEST SUMMARY

For spermatozoa, Fructose is the main source of energy.

The determination of fructose in the seminal fluid is considered an indicative test of the sperm vitality, as well as an index of the functionality of the seminal vesicles.

Low fructose values are characteristic in the presence of obstruction of the ejaculatory ducts, bilateral congenital absence of the vas deferens, partial retrograde ejaculation, androgenic deficiency.

### PRINCIPLE OF THE TEST

D-fructose, in presence of ATP, is transformed from Eschochinase (HK) in Fructose-6-phosphate. The Fructose-6-phosphate is transformed from Phospho-Gluco-Isomerase (PGI) in glucose-6-phosphate, that in its turn is transformed in 6-phosphogluconate from G6P-DH with formation of NADPH. NADPH formed in this reaction causes an increase of absorbance at 340 nm.

### SAMPLES

Sperm; use fresh sample.

### REAGENTS

Reagent A: Good's buffers > 10 mM ATP > 2 mM pH 7.8

Reagent B: NAD > 0.2 mM, pH 4.5.

Starter 1: HK > 300 U/l; G6PDH > 700 U/l.

Starter 2: PGI > 100 U/l.

Standard: Fructose 1 mg/ml.

Diluent: Detergent for Samples.

### MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatisation. Automatic Micropipette. Cuvette in optical glass or single use in optical polystyrene. Distilled / demineralised water.

### PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

### REAGENTS PREPARATION

Reagents, if not contaminated, are stable until the expiration date indicated on the package stored at 2-8°C.

### Working Reagent preparation

Mix 4 parts of Reagent A + 1 part of Reagent B (8 ml RA + 2 ml RB).

The working reagent is stable for 8 days at 2-8°C or 2 months at -20°C.

Freeze only one time. Do not repeat freezing. It's advisable to fractionate quantities to freeze in accordance with the number of daily tests.

Gently mix Starter 1 and Starter 2 before use to resuspend enzymes in solution.

### SAMPLE PREPARATION

Centrifuge sample at 3000 rpm for 10 minutes. Dilute 10 µl of sample with 600 µl of diluent.

### PROCEDURE

Kind of analysis:	End-Point
Reading time:	10, 20 minutes
Wavelength:	340 nm (334-365)
Temperature:	37°C

Pathlength: 1 cm  
Zero: Blank reagent

Reagents	Blank	Standard	Sample
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	20 µl	--	--
Standard	--	20 µl	--
Sample	--	--	20 µl
Starter 1	25 µl	25 µl	25 µl

Mix, wait the end of reaction (10 minutes) and measure absorbance of solutions (A<sub>1</sub>) against blank.

Starter 2	Blank	Standard	Sample
	25 µl	25 µl	25 µl

Mix, wait the end of reaction (further 10 minutes) and measure absorbance of solutions (A<sub>2</sub>) against blank.

### CALCULATION

Fructose (mg/ml)

$$\frac{A_2 (\text{sample}) - A_1 (\text{sample})}{A_2 (\text{standard}) - A_1 (\text{standard})} \times 61 \times 1$$

### EXPECTED VALUES

Fructose (mg/ml) 2 - 5

Every laboratory should establish own reference intervals in accordance with own population.

### NOTES

- As with any diagnostic procedure, if the results are incompatible with clinical presentation, the physician should confirm the data obtained using this test, with other clinical information.
- Only for IVD use.

### CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following kit is available on request:

### FERTILITY – CONTROL SET

#### FK00400 3 levels x 1 ml

Control solutions for determination of biochemistry parameters in seminal fluid

### TEST PERFORMANCE

#### Precision

Intra-assay (n = 10)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	351.25	4.25	1.21
Sample 2	167.12	2.87	1.72

Inter-assay (n = 10)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	352.58	5.08	1.45
Sample 2	169.74	3.41	2.01

### Methods comparison

A comparison with an available commercial method gave following results on 25 samples compared:

$$\text{Fructose LTA} = x$$

$$\text{Fructose competitor} = y$$

$$n = 25$$

$$y = 0,9784x - 0,0012 \text{ mg/dl} \quad r = 0,99831$$

### Linearity

The method is linear up to 1.5 mg/ml in diluted sample (equivalent to 90 mg/ml in whole sample).

### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

### PACKAGING

CODE FK00100	(100 TESTS)	
Reagent A	1 x 80 ml	(liquid)
Reagent B	1 x 20 ml	(liquid)
Starter 1	1 x 2.5 ml	(liquid)
Starter 2	1 x 2.5 ml	(liquid)
Standard	1 x 5 ml	(liquid)
Diluent	2 x 65 ml	(liquid)

### REFERENCES

Bergmeyer, H.U., Gruber, W., Gutmann, I. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, S. 1368-1371, Verlag Chemie, Weinheim, And (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) Vol.3, pp. 1323-1326; Verlag Chemie, Weinheim & Academic Press, Inc. New York And London.  
Beutler, H.O. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., Vol. VI, pp. 356-362, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel.  
Pasquinelli, Diagnostica e Tecniche di laboratorio Vol.1, p.1379.

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### SYMBOLS

	Only for IVD use
	Lot of manufacturing
	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

Mod. 01.06 (ver. 4.0 - 27/07/2018)

