Fructose Test

96 Tests

PHOTOMETRIC TEST FOR QUANTIFYING FRUCTOSE IN HUMAN SEMINAL PLASMA

Document reference: FP09 I29 R01 B.12 Update: 1/07/2020

IVD

Fructose standard contains 0.09% Na-Azide

For in vitro diagnostic use - Reagent for professional use only.

GENERAL INFORMATION

Fructose in semen reflects the secretory function of seminal vesicles. The purpose of the Fructose Test is to measure the amount of Fructose in human semen or seminal plasma. The Fructose Test may help in assessing the diagnosis and the management of male infertility.

TEST PRINCIPLE

Fructose reacts, in the presence of HCl under heat, with indole and produces a coloured complex which can be measured at a wavelength of 450-492nm.

MATERIAL INCLUDED IN THE KIT

- Reagent 1 - 50ml TCA solution
- Reagent 2 - 25ml Concentrated HCl (32%)
- Reagent 3 - 3ml Indole in methanol
- Reagent 4 - 25ml NaOH (0.5M)
- Fructose Standard - 10ml (5mg/ml)

A certificate of analysis and MSDS are available upon request or can be downloaded from the website (www.fertipro.com).

MATERIAL NOT INCLUDED IN THE KIT

Plate reader / photometer (with 450-492nm filter), pipettes and fresh tips, centrifuge tubes, titre plate, centrifuge (> 1000g), small reagent tubes or Eppendorf tubes, water bath or heat block.

SPECIMEN TYPE

Perform the test preferably on (frozen/thawed) semen plasma instead of the whole semen sample, especially in cases where the sample is not immediately analyzed (i.e. not within 3 hours after ejaculation). This, to avoid that spermatozoa metabolize fructose leading to an underestimation of fructose concentrations. Freeze seminal plasma when it cannot be tested at the same day.

METHOD

We recommend to view our demonstration video before you first start the test. Hereto, download the video via the link on our website, or scan barcode:

1. Allow the semen sample to liquefy at room temperature
2. Measure total semen(plasma) volume (e.g. with a sterile syringe)
3. Pipet 100µL of semen sample/seminal plasma into separate test tubes
4. Pipet 100µL of the Fructose standards (see below how to prepare the standards) into a test tube and treat like a semen sample
5. Add 500µL of Reagent 1 (TCA solution) to the samples and standards and mix
6. Centrifuge for 10 minutes at 1000g or more
7. Pipette 20µL of supernatant/standard from step 6 into an empty Eppendorf tube or small reagent tube
8. Blanc sample: pipette 20µL of purified water into an empty Eppendorf tube or small reagent tube
9. Add 200µL of Reagent 2 (HCl) to each tube

Remark: perform step 10-13 under a fume hood because Reagent 3 is toxic by inhalation (see warnings and precautions)

10. Add 20µL of Reagent 3 (indole) to each tube
11. Close or seal tubes and incubate for 30 minutes at 37°C in a water bath or fitting heat block (recommended), or 60 minutes at 37°C in a dry incubator
12. Add 200µL of Reagent 4 to stop the colour reaction
13. Pipette 200µL of sample into an empty well and read results at 450-492nm in a plate reader

FRUCTOSE STANDARDS

The kit contains a 5mg/ml Fructose solution. Prepare a standard curve with the following fructose concentrations:

Standard	Fructose standard	Water
5mg/ml	100µl	0µl
2.5mg/ml	50µl	50µl
1mg/ml	20µl	80µl

Note: Prepare the dilutions of the standard using purified water (e.g. distilled water).

INTERPRETATION

Download the Excel calculation sheet from our website and enter data in the sheet to calculate results:
https://fertipro.com/2019/11/07/fructose-test/

The measured value (OD) for the sample is plotted against the standard curve (using the 3 standards mentioned above) with the OD on the Y axes and the concentration of fructose on the X axes. To obtain total Fructose amounts, multiply the result with the total volume of the semen sample or seminal plasma. Normal values according to the WHO manual:

- 2.4mg/ejaculate or more
- 13µmol/ejaculate or more

Note: The standard curve is linear down to 0.5mg/ml, lower amounts of fructose cannot be measured accurately with this kit.
Low fructose in semen is characteristic of ejaculatory duct obstruction, bilateral congenital absence of the vas deference, partial retrograde ejaculation and androgen deficiency (WHO, 2010).

ASSAY PERFORMANCE PARAMETERS

Intra-assay CV: 8% (Repeatability)
Inter-assay CV: 13% (Total precision)

STORAGE

Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37°C). Store reagents between 2°C and 8°C. Keep away from (sun)light. Product can be used for 12 months after date of production.

The bottle with Reagent 2 (HCl) may show a mild colour change to orange or pink (the liquid remains colourless), this does not affect the test results.

WARNINGS AND PRECAUTIONS

Reagent 1 (TCA solution): Causes severe burns. Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately.

Reagent 2 (32% HCl solution): Causes burns. Irritating to respiratory system. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately. Never add water to concentrated HCl.

Reagent 3 (Indole in methanol): Harmful if swallowed. Avoid contact with skin and eyes. Highly flammable. Toxic by inhalation and if swallowed. **Always work under a fume hood when using this reagent.**

Reagent 4 (NaOH): Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you are unwell, seek medical advice immediately.

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens and reagent (gloves, lab vest, eye/face protection).

CALCULATION OF G-FORCES

The g-force of your centrifuge can be calculated using this formula:

$$g = 1.118 \times r \times rpm^2 \quad \text{or} \quad rpm = \text{Square root } \{g / (1.118 \times r)\}$$

r = radius of centrifuge in mm; rpm = rotations per minute / 1000

e.g.1

r = 100 mm rpm = 3000 rotations per minute

g = 1.118 x 100 x 9 = 1006g

e.g.2

r = 100 mm g = 1200g

rpm = SQR {1200 / (1.118 x 100)} = 3.28

= 3280 rotations per minute

BIBLIOGRAPHY

WHO laboratory manual for the Examination and processing of human semen - 5th Edition. World Health Organization, 2010.

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