EN

Fructose Test

Diagnostic kit for the measurement of fructose in human semen or seminal plasma

IVD

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MATERIAL INCLUDED

Catalogue number

FRUCTO Fructose Test - 96 tests

CUSTOMER-TECHNICAL SUPPORT

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CE 2797

Fructose Test

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

GENERAL INFORMATION

The secretion of the seminal vesicles constitutes the main fraction of the ejaculate. Seminal fructose concentration can be used as a marker of the seminal vesicular function. Low fructose in semen is characteristic for partial or complete ejaculatory duct obstruction, bilateral congenital absence of the vas deference, partial retrograde ejaculation and androgen deficiency (WHO, 2021; ASRM, 2015).
Hence, the Fructose Test can be used as an aid in the diagnosis and management of male infertility/subfertility.

INTENDED USE

The Fructose Test is a quantitative, non-automated, photometric and diagnostic kit for the measurement of fructose in human semen or seminal plasma (fresh or frozen).
The Fructose Test may help in assessing the diagnosis and management of male infertility as fructose in semen reflects the secretory function of seminal vesicles.

A maximum of 44 samples can be tested with the reagents in the Fructose Test when samples are tested in duplicate in one test run.

TEST PRINCIPLE

Under the influence of heat and low pH, fructose reacts with indole and forms a coloured complex, which absorbs light at a wavelength of 450-492nm that can be measured with a spectrophotometer.

MATERIAL INCLUDED WITH THE TEST

- 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).

MATERIALS REQUIRED, BUT NOT PROVIDED

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

METHOD

Scan barcode (or download link on www.fertipro.com) to view the demonstration video.

Specimen collection and preparation
Standard semen collection containers should be used, typically in polypropylene and sperm survival/sperm motility tested, when semen is collected by masturbation. Non semen-toxic plastic condoms should be used when semen collection by masturbation is not possible.

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) or when sperm count is high. This to avoid that spermatozoa metabolize fructose leading to an underestimation of fructose concentration. Freeze seminal plasma or semen sample (at -20 °C without cryopreservation medium) when it cannot be tested at the same workday.

Reagent preparation
Do not use the product if seal of the bottles is opened or defect when the kit is delivered.

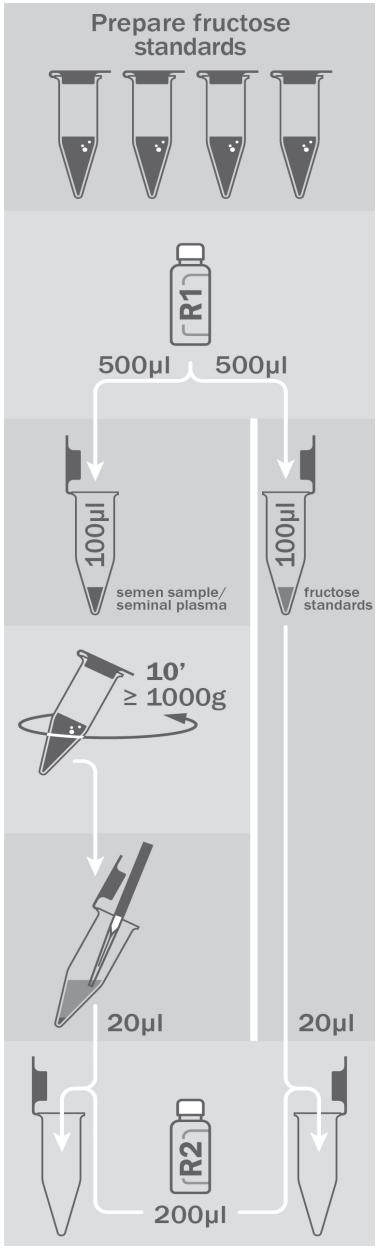
All reagents are ready to use. Allow to adjust to room temperature before use.

Sample preparation
1. Allow the semen sample to liquefy at room temperature.
2. Measure total semen (plasma) volume (e.g. with a syringe).

Method Fructose Test
Recommendations:
1. Prepare standard curve and samples in duplicate.
2. Prepare standard curve with a concentration range of 0 to 5mg/ml fructose using Fructose Standard (5mg/ml) and purified (e.g. distilled) water:

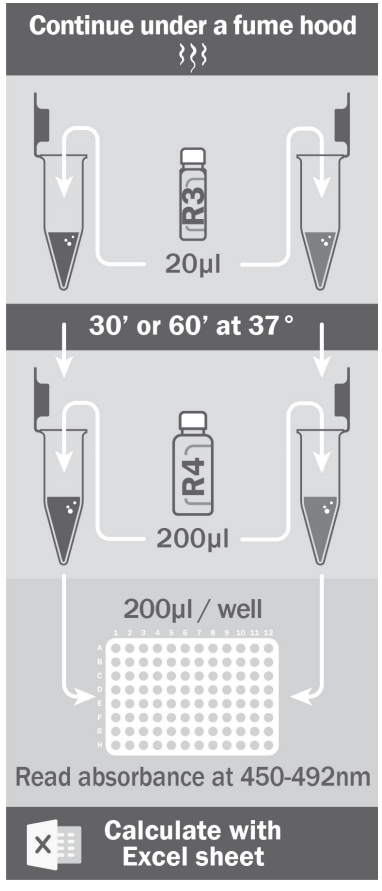
Standard	Fructose Standard	Water
5mg/ml	250 µl	0µl
2.5mg/ml	125 µl	125 µl
1mg/ml	50 µl	200 µl
Blank	-	250 µl

Graphic presentation of the protocol:



Clarification:

1. Pipet 100µL of semen sample/seminal plasma and 100µl of the fructose standards prepared in step 2 into separate test tubes (perform this preferably in duplo);
2. Add 500µL of Reagent 1 (TCA solution) to the samples and standards and mix;
3. Centrifuge the samples for 10 minutes at ≥ 1000g;
4. Carefully pipette 20µL of supernatant/ standard into an empty test tube. Avoid contact of pipette tip with the precipitates!
5. Add 200µL of Reagent 2 (HCl) to each tube;



From this step work in a fume hood (Reagent 3 is toxic by inhalation)

6 Add 20µL of Reagent 3 (indole) to each tube and mix;
7 Close or seal tubes and incubate for 30 minutes at 37°C in a water bath or fitting heat block (recommended), or for 60 minutes at 37°C in a dry incubator;
8 Add 200µL of Reagent 4 to stop the colour reaction;
9 Pipette 200µL of sample / standard into an empty well of microtiter plate and read results at 450-492nm in a plate reader/photometer;
10 After each individual test, discard all used reagents and materials.

INTERPRETATION

1 Average the duplicate reading for each standard and sample;
2 Subtract the mean absorbance value of the blank from all standard and sample readings. This is the corrected absorbance;
3 Plot the corrected absorbance values for each standard as a function of the fructose concentration of the standards;
4 Calculate linear regression based on your standard curve data; Coefficient of determination (R^2) should be ≥0.98;
5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$\text{Fructose concentration of samples (mg/ml)} = \frac{OD\ \text{sample}_{corrected} - \text{intercept}}{\text{slope}}$$

6 To obtain total fructose amount (mg/ejaculate), multiply the result with the total volume of the semen sample or seminal plasma;
7 A value of ≥ 3.0 mg fructose /ejaculate obtained with the Fructose Test is considered as a normal value.

Note: *For quick data analysis, download the Excel calculation sheet from our website and enter data in the sheet to calculate results:*

LIMITATIONS OF THE METHOD

The Fructose Test is an aid in the diagnosis of male infertility and, as for other biological tests, interpretation of the results must be performed within the framework of clinical findings and data of history taking. The Fructose Test can determine fructose levels between 0.5-5 mg/ml.

PERFORMANCE CHARACTERISTICS

Repeatability and reproducibility: CV_{intra} < 15%, CV_{inter} < 15%
Measuring range: 0.5-5 mg/ml
Cut-off: 3.0 mg fructose / ejaculate

STORAGE/DISPOSAL

- Fructose Test is stable for 12 months from the date of manufacture (even after opening).
- Do not use the product after expiry date.
- Store reagents between 2°C and 8°C.
- Keep away from (sun)light.
- Suitable for transport or short term exposure at elevated temperatures (up to 5 days at 25°C).
- Do not freeze.
- The reagents need to be disposed in accordance with the local regulations for disposal of medical devices.
- 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.
Fructose standard contains 0.09% Na-Azide.

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).
Any serious incident (as defined in the European In Vitro Diagnostic Medical Device Regulation 2017/746) that has occurred should be reported to FertiPro NV and, if applicable, to the competent authority of the EU Member State in which the user and/or patient is established.

BIBLIOGRAPHY

WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021
ASRM. (2015). Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril, 103(3), e18-25. doi:10.1016/j.fertnstert.2014.12.103

SYMBOLS GLOSSARY

Symbols as defined in ISO 15223	
REF	Catalogue number
LOT	Batch code
	Consult instructions for use
	Manufacturer
IVD	In Vitro Diagnostics
°C	Temperature limit
	Use-by date
	Keep away from sunlight
Symbol as defined in IVDR 2017/746	
CE 2797	CE marking by Notified Body 2797
Symbol as defined in regulation (EC) No. 1272/2008 [CLP]	
	GHS08 Serious health hazard
	GHS02 Flammable liquid
	GHS06 Acute toxicity
	GHS07 Health hazard
	GHS05: Skin corrosion/irritation
	GHS09 Hazardous to the aquatic environment

Other languages can be downloaded on our website (www.fertipro.com)