CITRIC ACID IN SEMINAL FLUID

UV method for determination of Citric Acid in semen

TEST SUMMARY

Seminal Citric Acid represents an index of prostatic function. High concentrations can be found in cases of prostate infection.
Low concentrations are found in cases of obstructive disease accompanied by low testosterone levels.

PRINCIPLE OF THE TEST

The Citric Acid (citrate) is changed in oxalacetate and acetate by CL (Citrate lyase).
In presence of Malate-dehydrogenase (MDH) and Lactate-dehydrogenase (LDH), the oxalacetate and pyruvate (decarboxilated product of oxalacetate), are trasformed in L-Malate and L-Lactate, giving oxidization of NADH in NAD^+.
The formation of NAD^+ causes a diminution of absorbance at 340 nm.

SAMPLES

Sperm. Stability 2 days at 2-8 °C

REAGENTS

Buffer:	Good buffer > 10 mM pH 7.8; LDH 500 U/l.
Substratum/Enzyme:	MDH > 350 U/l; NADH > 0.1 mM.
Starter:	CL > 300 U/l.
Standard:	Citric Acid 0.25 g/l.
Diluent:	Samples detergent.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Physiologic solution.

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

Dissolve a vial of Substratum with 20 ml of Buffer mixing gently till dissolution to avoid foaming formation.
Add 0.5 ml of buffer to vial of Starter, mix gently to avoid foaming formation.
Reagents are stored at 2-8°C until the expiration date stated on the label.
The Substratum reconstituted is stable for 10 days at 4°C, for 1 month at -20°C.
The starter reconstituted is stable for 24 hours at 4°C or 1 month 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.

SAMPLE PREPARATION

Centrifuge the sample at 3000 rpm for 10 minutes.
Dilute 20 µl of sample with 1200 µl of diluent.

PROCEDURE

Method:	End-Point
Wavelength:	340 nm (334-365)
Temperature:	37°C
Pathlength:	1 cm
Zero:	Blank reagent

Reagents	Blank	Standard	Sample
Substratum	1000 µl	1000 µl	1000 µl
Standard	--	30 µl	--
Sample	--	--	30 µl
Distilled water	30 µl	--	--
Starter	25 µl	25 µl	25 µl

Mix, wait the end of the reaction (10 minutes) and measure absorbance of solutions (A) against blank.

CALCULATION

Citric Acid (g/l)

$$\frac{A\ (sample)}{A\ (standard)} \times 61 \times 0.25$$

REFERENCE INTERVALS

Citric Acid (g/l) 3,50 - 6,70

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

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- 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 (g/l)	SD (g/l)	CV%
Sample 1	3.02	0.0189	0.63
Sample 2	4.64	0.0200	0.43

Inter-assay (n = 10)	Mean (g/l)	SD (g/l)	CV%
Sample 1	3.03	0.0462	1.53
Sample 2	4.61	0.0629	1.36

Linearity
The method is linear until 24 g/l.
If the value is higher than 24 g/l, it's advisable to dilute the sample 1:4 with physiologic solution and repeat the test, multiplying the result by 4.

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

Citric Acid LTA = x
Citric Acid competitors = y
n = 25

y = 1,00093x + 0,00703 r = 0,9960

WASTE DISPOSAL

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

PACKAGING

CODE FK00250	**(100 TESTS)**	
Buffer	1 x 100 ml	(liquid)
Substratum	5 x 20 ml	(liophile)
Starter	5 x 0.5 ml	(liophile)
Standard	1 x 10 ml	(liquid)
Diluent	2 x 65 ml	(liquid)

*Buffer supplied in excess sufficient for the reconstitution of both the substrate and the starter.

REFERENCES

1. Möllering, H.& Gruber, W. (1966) Determination of citrate with citrate lyase, Anal. Biochem. 17, 369-376.
2. Dagley, St.(1974) in Methoden der enzymatischen Analyse (Bergmeyre, H.U., Hrsg.) Bd. 2, S. 1607-1611; Verlag Chemie Weinheim ana (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 3 pp. 1562-1565, Verlag Chemie, Weinheim, Academic Press, Inc., New York and London.
3. Pasquinelli, Diagnostica e Tecniche di laboratorio Vol.1, p.1379.

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SYMBOLS

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

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