CITRIC ACID

Enzymatic colorimetric determination of citric acid in sperm

TEST SUMMARY

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

Urine, 24 hours urine. Stability 4 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:	Cleaner samples

MATERIAL REQUIRED BUT NOT SUPPLIED

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

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 l of sample with 1200 Diluent..

PROCEDURE

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

Reagents	Blank	Standard	Sample
Substratum Standard Sample Distilled water	1000 µl -- -- 30 µl	1000 µl 30µl -- --	1000 µl -- 25 µl --
Mix and incubate for 3 minutes at 37°C, read absorbances (A_1) against blank			
Starter	25 µl	25 µl	25 µl
Mix, wait the end of the reaction (10 minutes) and measure absorbance of solutions (A_2) against blank			

CALCULATION

Citric Acid (g/l)

$$\frac{[A_2 \text{ (sample)} - A_1 \text{ (sample)}]}{[A_2 \text{ (standard)} - A_1 \text{ (standard)}]} \times 61 \times 0.25$$

EXPECTED VALUES

Acido Citrico 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 control solutions are available on request:

6 x 5 ml
Control set Oxalic acid / Citric acid
(Normal values – Pathologic values)

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 up to 0.4 g/l.
If the value is higher than 0.4 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 Intermedical = x
Citric Acid competitors = y
n = 25

y = 1.00093 + 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

CIT	**(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)

REFERENCES

Möllering, H.& Gruber, W. (1966) Determination of citrate with citrate lyase, Anal. Biochem. 17, 369-376.
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.
MedlinePlus Medical Encyclopedia: Citric acid urine test, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894.

MANUFACTURER

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SYMBOLS

IVD Only for IVD use

Storage temperature interval

Expiration date

LOT Lot of manufacturing

REF Code number

Read the directions