CITRIC ACID IN URINE

Enzymatic colorimetric determination of citric acid in urine

TEST SUMMARY

Citric acid is the main inhibitor of calcium crystallization and crystalline growth, that is, the process by which kidney stones are formed.
It combines with calcium in the lumen of the renal tubules to form a soluble complex thereby reducing the availability of calcium to form oxalate crystals with subsequent formation of stones.
Urinary levels depend on diet and base acid balance.
A reduced urinary excretion of citrate (IPOCITRATURIA) represents a risk factor for the formation of stones, in association with an increased excretion of calcium, oxalates, urates.

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 reaction is kinetically monitored at 340 nm through the decrease in absorbance resulting from the oxidation of NADH to NAD^+, proportional to the activity of citric acid in the sample.

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: MDH > 350 U/l; NADH > 0.1 mM.
H302 – Harmful if swallowed
H412 – Harmful to aquatic life with long lasting effects.

Starter: CL > 300 U/l.
H301 – Toxic if swallowed
H411 – Toxic to aquatic life with long lasting effects

Standard: Citric Acid 0.25 g/l.

For more warnings and precautions, see the Product Safety Data Sheet (MSDS)

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.

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

The sample must be limpid by centrifugation or filtration.

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	--	25 µl	--
Sample	--	--	25 µl
Distilled water	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 and keep incubating at 37°C. Wait for the end of the reaction (10 minutes) and measure the absorbance of the solutions (A_2) by zeroing against blank.			

CALCULATION

Citric Acid (g/l)

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

EXPECTED VALUES

Citric acid mg/24 hours 320 - 1240

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 advisable to carry out an internal quality control. For this purpose, the following control solutions are available on request, which must be treated as if these were samples:

CC02430 6 x 5 ml (2 levels)
Control set Oxalic acid / Citric acid

Each laboratory must have its own Quality System and establish corrections if the controls do not respect the tolerances.

TEST PERFORMANCE

Precision

Intra-assay (n = 20)	Mean (g/l)	SD (g/l)	CV%
Sample 1	0.170	0.002	1.38
Sample 2	0.580	0.004	0.77

Inter-assay (n = 20)	Mean (g/l)	SD (g/l)	CV%
Sample 1	0.169	0.004	2.29
Sample 2	0.575	0.009	1.63

Sensibility/limit of detection
The method is able to discriminate until 0.02 g/l.

Linearity
The method is linear up to 2 g/l.
If the value is higher than 2 g/l, it is recommended to dilute the sample with saline solution and repeat the test multiplying the result by the dilution factor.

Methods comparison
A comparison with an available commercial method gave following results on 50 samples compared:

Citric Acid LTA = x
Citric Acid competitors = y
n = 50

y = 1,00381x - 0,0028 mg/dl r = 0,99827

WASTE DISPOSAL

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

PACKAGING

CODE CC00150	(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)

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

CODE CC00155		
Buffer	4 x 100 ml	(liquid)

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. MedlinePlus Medical Encyclopedia: Citric acid urine test, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894.

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SYMBOLS

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

NOTE: MODIFICATIONS HIGHLIGHTED WITH GRAY BACKGROUND