OXALIC ACID

Enzymatic colorimetric determination of oxalic acid in urine

TEST SUMMARY

Oxalic acid, produced as a waste from metabolism or taken from food sources, is normally eliminated via the kidneys.
When combined with calcium it gives rise to calcium oxalate, a salt that comes in the form of crystals and tends to precipitate easily, giving rise, if in excess, to deposits in the urinary tract that can cause tubulo-interstitial nephropathy, nephrocalcinosis, kidney stones.
A decrease in oxalate excretion in the urine may be associated with hyperglycaemia and hyperglycinuria.
An increase in oxalate excretion could be caused by the type of diet with an increase in the ingestion of oxalate precursors or oxalate-rich foods, metabolic defects, or fat malabsorption caused by the absorption of oxalate in the presence of several gastrointestinal disorders.

PRINCIPLE OF THE TEST

Oxalic acid is transformed by oxalate oxidase into carbon dioxide and hydrogen peroxide.
This, reacting in a Trinder system in the presence of peroxidase (POD), forms a blue/violaet colored compound.
The intensity of the color produced is directly proportional to the concentration of oxalic acid in the sample.

SAMPLES

24 hours urine. Record the volume in liters.
If analysis is not performed immediately, acidify urine of 24 hours with 10 ml of HCl concentrated.
Stability: 7 days at 2-8°C.

REAGENTS

Reagent 1	Succinic buffer pH 3.80; 3-(Dimethylamino) Benzoic acid, preservatives and stabilizers.
Reagent 2	Succinic buffer pH 3.80; preservatives and stabilizers
Reagent 3	Oxalate oxydase, peroxydase, preservatives and stabilizers.
Standard	Oxalate 0.5 mM, preservatives and stabilizers.
Sample diluent	Buffer pH 7.0 EDTA, preservatives and stabilizers.
Purification tubes	Active coal.

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.

PRECAUTION

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

All reagents are stable until expiration date on label if stored at 2-8°C.
Reconstitute Reagent 3 with 2 ml of distilled water.
Reagent 3 reconstituted is stable 1 month at 2-8°C.
Is possible to pre-mix Reagent 1 with Reagent 2 in equal parts. Stability: 1 month at 2-8°C.

The Standard is ready to use, it must not follow the procedure with the diluent and the purification tubes shown below, which only applies to the samples and controls of the LTA CC02430 kit (supplied separately).

SAMPLES PREPARATION

Mix 1 ml of urine with 1 ml of sample diluent.
Control pH and if needed, correct with HCl 1N or NaOH 1N up to obtain a value between 5.0 and 7.0.
Pour out this mixture in a purification tube.
Shake very well for 5 minutes with continuous inversion or using a rotating stirrer. Centrifuge or filter.

PROCEDURE

Method:	End point
Reading time:	10 minutes
Wavelength:	590 nm(580-600)
Temperature:	25, 30, 37°C
Lightpath:	1 cm
Zero:	Blank reagent

Reagents	Blank	Standard	Sample
Reagent 1	500 µl	500 µl	500 µl
Reagent 2	500 µl	500 µl	500 µl
Distilled water	50 µl	--	--
Standard	--	50 µl	--
Sample	--	--	50 µl
Reagent 3	100 µl	100 µl	100 µl

Add at the end, Reagent 3; shake very well and incubate for 10 minutes. Bring to naught against blank and read extinctions.

CALCULATION

Oxalic acid (mM)

(A sample/A standard) x 0.5 x 2

Oxalic acid (mmol/24 h)

(A sample/A standard) x 0.5 x 2 x l of urine

EXPECTED VALUES

Men:
0.08 - 0.49 mmol/24h (7 - 44 mg/24h)

Women:
0.04 - 0.32 mmol/24h (4 - 31 mg/24h)

Children:
0.14 - 0.42 mmol/24h (13 - 38 mg/24h)

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

NOTE

- If you want to obtain value worded in mg, multiply results in mmol by 90.
- 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's advisable to perform an internal quality control. In order to do this, on request are available the following control solutions:

CC02430 **6 x 5 ml**
Control Set Oxalic acid / Citric acid
(Normal value – Pathologic value)

TEST PERFORMANCE

Precision

Intra-assay (n = 15)	Mean (mmol/24h)	SD (mmol/24h)	CV%
Sample 1	0.105	0.004	4.18
Sample 2	1.048	0.025	2.43

Inter-assay (n = 20)	Mean (mmol/24h)	SD (mmol/24h)	CV%
Sample 1	0.106	0.007	6.91
Sample 2	1.041	0.050	4.84

Linearity
Method is linear up to 1 mM.

Methods comparison
A comparison with an available commercial method gave following results on 40 samples compared:
Oxalic acid LTA = y
Oxalic acid competitors = x
n = 40
y = 0,985x + 0,008 r = 0,9978

Interferences
High concentrations of Ascorbic acid interfere with the test determination.
The concentration of oxalic acid in the urine can be influenced by the type of diet.

WASTE DISPOSAL

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

PACKAGING

CODE CC02400	**(20 TESTS)**
Reagent 1	1 x 10 ml (liquid)
Reagent 2	1 x 10 ml (liquid)
Reagent 3	1 x 2 ml (powder)
Standard	1 x 5 ml (liquid)
Sample diluent	1 x 20 ml (liquid)
Purification tubes	20

CODE CC02410	**(100 TESTS)**
Reagent 1	1 x 50 ml (liquid)
Reagent 2	1 x 50 ml (liquid)
Reagent 3	5 x 2 ml (powder)
Standard	1 x 5 ml (liquid)
Sample diluent	1 x 100 ml (liquid)
Purification tubes	100

CODE CC02415 (whitout Purification tubes)	**(100 TESTS)**
Reagent 1	1 x 50 ml (liquid)
Reagent 2	1 x 50 ml (liquid)
Reagent 3	5 x 2 ml (powder)
Standard	1 x 5 ml (liquid)
Sample diluent	1 x 100 ml (liquid)

CODE CC02420	**(Extraction kit)**
Sample diluent	1 x 100 ml (liquid)
Purification tubes	100

CODE CC02421	
Sample diluent	4 x 100 ml (liquid)

REFERENCES

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Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AACC Press (2000).

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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
	Biologic risk

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