OROTIC ACID

Colorimetric determination of orotic acid and orotodine in urine

TEST SUMMARY

Orotic Acid is an intermediate in the pathway of pyrimidine synthesis. It increase considerably in urine of homozygous and heterozygous subjects for orotic aciduria, rare hereditary desease. Furthermore is possible verify levels elevation in some physiological situations, including pregnancy in women receiving hyperalimentation following allopurinol and azothiaprine therapy, and in some urea cycle disorders.
In orotic aciduria the metabolic block consists in the lack, genetically determined, of orotidine phosphoribosyltransferase and orotodine decarboxylase. This last enzyme is inhibited also by the acid 6-azouridline, metabolite of antineoplastic 6-azouridine, which can produces in those subjects a kind of orotic aciduria, obviously not on genetically based.
Even allopurinol, used in gout treatment, can reacts forming a nucleoside which hinibites the oroticodecarboxylase determining orotico aciduria and orotidine.
In diseases linked to the urea cycle the orotic acid excretion is normal in carbamyl phosphate sythetase deficiency, but it results elevated in patients with ornithine transcarbamylase deficiency, citrullinemia, argininosuccinic aciduria, argininemia and lysinurc protein intollerance.

PRINCIPLE OF THE TEST

Orotic acid has been separated from the interferences present in urine with a treatment as the resin ionic exchange. After collection into eluate, is brominated and so reduced to barbituric acid which reacts with p-dimethylaminobenzaldehyde (DAB) forming acid 5-(p-dimethyilamino-benziliden) barbituric.

SAMPLES

Urine. Stability 3 days at 2-8°C, 1 month at –20°C.

REAGENTS

Buffer A:	Trichloroacid; potassium bromine.
Buffer B:	Bufferphosphate; sodium bromine.
Ascorbic acid:	Ascorbic acid.
DAB:	Dimethylaminobenzaldehyde (DAB).
Standard:	Watery solutions as knowed title of orotic acid (0,25 - 0,5 - 1 - 2 - 3 mg/dl).
Resin:	Tubes containing resin at ionic exchange.

REAGENTS PREPARATION AND STORAGE

Reagents are ready to use and have to be stored at 2-8°C until expiration date on label.
Buffer A can produce a precipitants of potassium bromine that are redissolved by water-bath.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general “Good Laboratory Practice” (GPL) guidelines.

SAMPLE PREPARATION

Remove the filter to the tube containing resin and add 2 ml of sample or standard. Put the filter at some centimetres from the liquid border e resuspend for inversion more time and shake at least for 5 minutes. Press the filter to the bottom and use the eluate.

PROCEDURE

Kind of analysis:	End-point
Reading time:	60 minutes
Wavelength:	480 nm (470 - 490)
Temperature:	Room temperature
Lightpath:	1 cm

Reagents	Sample Blank	Sample	Standard Blank	Standard
Eluate	400 µl	400 µl	400 µl	400 µl
Buffer A	600 µl	600 µl	600 µl	600 µl
Buffer B	--	1000 µl	--	1000 µl
Ascorbic acid	200 µl	--	200 µl	--
Shake and wait 30 minutes at room temperature.				
Buffer B	1000 µl	--	1000 µl	--
Ascorbic acid	--	200 µl	--	200 µl
Shake and wait 5 minutes at room temperature.				
DAB	1000 µl	1000 µl	1000 µl	1000 µl
Shake and wait 60 minutes at room temperature in the dark.				

RESULTS

Calculate ΔA : $A_{sample} - A_{blank}$

ΔA obtained from standards form a calibration curve reporting the absorbances (ordinate) and the relatives standards concentrations (abscissa).
Samples concentration is determined by absorbances values by means of the calibration curve.

EXPECTED VALUES

Age	mM orotic acid / creatinine moll
< 2 weeks	0.13 – 6.33
2 weeks - 1 year	0.20 – 4.72
1 anno - 10 anni	0.05 – 3.54
> 10 years	0.14 – 2.35

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’s advisable to perform an internal quality control. In order to do this, on request are available the following control solutions:

SN00630 **2 x 10 ml**
OROTIC ACID - Control Set (2 levels)

TEST PERFORMANCE

Precision

Intra-assay (n = 10)	Mean	SD	CV%
Sample 1	0.26	0.02	7.6
Sample 2	1.03	0.03	2.8

Inter-assay (n = 10)	Mean	SD	CV%
Sample 1	0.27	0.02	7.4
Sample 2	1.16	0.036	3.0

Sensibility/limit of detection
LTA method is sensible until the limit of 0.125 mg/dl.

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 33 samples:

n = 33 LTA = y Competitor = x

y = 0,96587x - 0,00352 r = 0,98801

WASTE DISPOSAL

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

PACKAGING

CODE SN00600

Buffer A	4 x 20 ml
Buffer B	1 x 110 ml
Ascorbic acid	1 x 25 ml
DAB	1 x 110 ml
Standard	5 values x 7 ml
Tubes with resin	50 pezzi

REFERENCES

M.L. Harris and V.G. Oberholzer Clin Chem. 26, 3 473 (1980).

MANUFACTURER

LTA s.r.l.
Via Milano 15/F
20060 Bussero (Milan) ITALY
Tel: ++39 02 95409034
Fax: ++39 02 95334185
e-mail: info@ltaonline.it
Website: http://www.ltaonline.it

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

Mod. 01.06 (ver. 3.3 - 05/12/2005)

CE