URIC ACID MR CE

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For *in vitro* diagnostic use only			

URIC ACID MR

Enzymatic colorimetric method

ENDPOINT

PRINCIPLE

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of ADPS* and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of uric acid in the sample.[1,2]

$$\text{Uric acid} + O_2 + 2\ H_2O \xrightarrow{\text{URICASE}} \text{Allantoin} + H_2O_2$$

$$\text{4-AA} + \text{ADPS} \xrightarrow[\text{POD}]{H_2O_2} \text{Quinoneimine} + 4\ H_2O$$

***N*-ethyl-N-sulphopropyl-m-anisidine**

REAGENT COMPOSITION

R1 **Monoreagent**. PIPES buffer 100 mmol/L pH 7.0, uricase > 50 U/L, peroxidase > 1 KU/L, 4-aminoantipyrine 0.32 mmol/L, ADPS 0.33 mmol/L, non-ionic tensioactives 2 g/L (w/v). Biocides.

CAL **Uric acid standard**. Uric acid 6 mg/dL (357 µmol/L). Organic matrix based primary standard. Traceable to SRM 909.

STORAGE AND STABILITY

Store at 2-8ºC.
The Monoreagent and Standard are stable until the expiry date stated on the label.
Discard the reagent if presents an absorbance over 0.300 at 550 nm against distilled water or if it fails to recover the declared values of control sera.

REAGENT PREPARATION

The Monoreagent and Standard are ready-to-use.

SAMPLES

Whenever possible medication should be suspended 12 hours before sample collection.
Hemolysis-free serum, EDTA or heparinized plasma, and urine (see Notes).
Uric acid in serum or plasma is stable up to 5 days at 2-8ºC and for 6 months at –20ºC.

INTERFERENCES

- Highly icteric or hemolytic samples should be discarded.
- Lipemic samples may require a blank correction. Use the same volume of sample with isotonic saline in the place of the reagent.
- Sedatives and analgesics with strong reducing properties, like buscapine or high dosis of ascorbic acid (Vitamin C), compete with peroxidase for the hydrogen peroxide giving low results.
- Other interfering compounds have been described.[3]

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 550 ± 10 nm.
- Constant temperature incubator set at 37ºC.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes:

TUBES	Blank	Sample	Standard
Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	25 µL	–
Standard	–	–	25 µL

3. Mix and let stand the tubes 10 minutes at room temperature or 5 minutes at 37ºC.
4. Read the absorbance (A) of the samples and the standard at 550 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

CALCULATIONS

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = \text{mg/dL uric acid}$$

Samples with concentrations higher than 20 mg/dL should be diluted 1:5 with saline and assayed again. Multiply the results by 5.

If results are to be expressed as SI units apply:
mg/dL x 59.5 = µmol/L

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REFERENCE VALUES[4]

Serum, plasma

Men	3.5-7.2 mg/dL (208-428 µmol/L)
Women	2.6-6.0 mg/dL (155-357 µmol/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of uric acid. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of uric acid. Assayed.

CLINICAL SIGNIFICANCE

Uric acid is the major product of the catabolism of purine nucleosides (adenosine and guanosine) from the purine metabolism pathway. Purines may be synthesized endogenously from the breakdown of nucleic acids or may be obtained from sources as diets in which nucleic acids are present.

An abnormal increase in the level of uric acid in the circulation above 7.0 mg/dL (0.42 mmol/L) is referred to as *hyperuricemia*, being the gout the major form of the ailment resulting in the deposition of urates in the soft tissues, especially in the joint areas. Increased levels may be also found associated with leukemia, toxemia of pregnancy and severe renal impairment.

Less common are the cases of *hypouricemia* where the concentration of uric acid is below 2.0 mg/dL (0.12 mmol/L). These cases are usually secondary to cases of hepatocellular disease, renal reabsorption defect, or overtreatment with uricosuric drugs used in the treatment of hyperuricemia.

NOTES

- Ascorbic acid disappears from serum samples in less than 90 minutes.[5]
- Uric acid in urine may be assayed on fresh random or timed (24-h) samples. To prevent urate precipitation specimens are brought to pH > 8 with 0.01N NaOH. Dilute urine 1:100 with distilled water before the analysis.
- Men and women on normal diets present values < 400-800 mg/24-h.[4]

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 25 mg/dL
- ***Precision***

mg/dL	Within-run			Between-run		
Mean	4.8	8.4	13.7	4.8	8.3	13.6
SD	0.04	0.08	0.7	0.05	0.07	0.14
CV%	0.9	0.1	0.6	1.0	0.8	1.0
N	10	10	10	10	10	10

Replicates: 10 for each level. Replicates: 10 for each level
Instrument: CECIL CE 2021 for 10 days.

- ***Sensitivity.*** Using a 1:40 sample/reagent at 550 nm, 1 mg of uric ac. will produce a net absorbance of approximately 0.016.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 30 r = 0.995 y = 0.980 - 0.135

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