

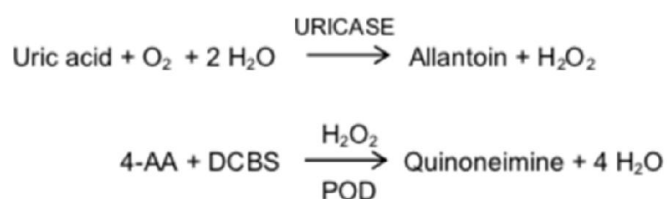
## Experiment no.: 2.

**Experiment name:** Determination of uric acid in the blood serum.

### The aim of the Experiment:

Determination of uric acid in the blood *Enzymatic colorimetric method* ENDPOINT.

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of dichlorophenol sulphonate (DCBS) 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.<sup>1,2</sup>



### Equipment and materials used in the Experiment:

- – Photometer or colorimeter capable of measuring absorbance at  $520 \pm 10$  nm.
- – Constant temperature incubator set at  $37^\circ\text{C}$ .
- – Pipettes to measure reagent and samples

### Property of the machine:

Normal UV-Vis spectrophotometer:

### Machine usage:

- Wavelength set up step.
- Blank against the solvent solution using a proper cuvette.
- Reach O.D.

### Experiment procedure or protocol:

1. Bring reagents and samples to room temperature. 2. Pipette into labelled tubes:			
TUBES	Blank	Sample	CAL. Standard
R1. Monoreagent Sample CAL.Standard	1.0 mL --	1.0 mL 20 µL –	1.0 mL – 20 µL
3. Mix and let the tubes stand 10 minutes at room temperature or 5 minutes at 37 °C. 4. Read the absorbance (A) of the samples and the standard at 520 nm against the reagent blank.			
The color is stable for at least 30 minutes protected from light.			

**R1 Monoreagent.** Phosphate buffer 100 mmol/L pH 7.8, uricase > 0.5 KU/L, peroxidase > 0.5 KU/L, ascorbate oxidase > 1 KU/L, 4-aminoantipyrine 0.5 mmol/L, DCBS 2 mmol/L, non-ionic tensioactives 2 g/L (w/v). Biocides.

**R2 Uric acid standard.** Uric acid 6 mg/dL (357 µmol/L). Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 909b.

### CALCULATIONS

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

Samples with concentrations higher than 30 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:  $\text{mg/dL} \times 59.5 = \mu\text{mol/L}$

### Experiment data and results:

Serum, plasma

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

**Conclusion:**

- Where is uric acid synthesized from?
- How does it affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?