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.[1,2]

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

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

Equipment and martials used in the Experiment:

- − Photometer or colorimeter capable of measuring absorbance at 520 ± 10 nm.
- − Constant temperature incubator set at 37ºC.
- − 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 ºC.
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 Sample/ A Standard X C Standard = 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: mg/dL x 59.5 = µmol/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?