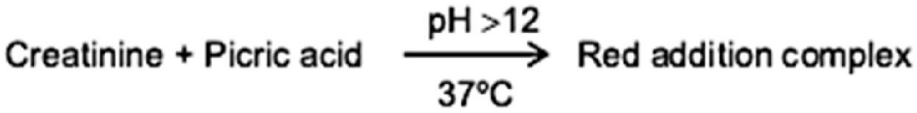
Experiment no.: 1.

Experiment name: Determination of Creatinine in the blood serum.
The aim of the Experiment:

Determination of Creatinine in the blood using Kinetic colorimetric method.

This procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.



Equipment and martials used in the Experiment:

- Photometer or colorimeter with a thermostatted cell compartment, able of reading at 510 ± 10 nm.
- – Constant temperature incubator set at 37 ^{o}C.
- – Stopwatch, strip-chart recorder or printer.
- – Cuvettes with 1-cm pathlength.
- – Pipettes to measure reagent and samples.

REAGENT PREPARATION

- R1 COMPOSITION Picric acid. Picric acid 25 mmol/L
- R2 Alkaline buffer. Phosphate buffer 300 mmol/L pH 12.7, SDS 2.0 g/L (w/v). Xi R:36/37/38
- Cal Creatinine standard. Creatinine 2 mg/dL (177 μmol/L). Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 914a.
- Working reagent. Mix 1 volume of R1 + 1 volume of R2. Stable for 1 week at room temperature, stored tightly closed and protected from light.

Property of the machine:

Normal UV-Vis spectrophotometer:

Machine usage:

1- Wavelength set up step.

2- Blank against the solvent solution using a proper cuvette.

3- Reach O.D.

Experiment procedure or protocol:

1. Preincubate working reagent, samples and standard to reaction temperature (37 ^{o}C).

2. Set the photometer to 0 absorbance with distilled water. 3. Pipette into a cuvette.

Working reagent	1.0 ml
Sample or Standard	100 ml

4. Mix gently. Insert cuvette into the temperature-controlled instrument and start stopwatch.

5. Record absorbance at 510 nm after 30 seconds (A_1) and after 90 seconds (A_2) of the sample or standard addition.

Experiment data and results:

Reference value:

Plasma

Men	0.70 - 1.20 mg/dL (62 - 106 μmol/L)
Women	0.50 - 0.90 mg/dL (44 - 80 μmol/L)
Urine	
Men	14 - 26 mg/Kg/24-h (124 - 230 μmol/Kg/24-h)
Women	11 - 20 mg/Kg/24-h (97 - 117 μmol/Kg/24-h)
Clearance Test	
Men	97 - 137 mL/min
Women	88 - 128 mL/min

Conclusion:

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