**Estimation of creatinine in urine**

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**Aim:** To estimate creatinine in urine.

**Principle:** Creatinine develops an orange red colour when treated with picric acid in the presence of strong alkali. This colour is due to the formation of creatinine picrate and measured at 460 nm.

Normal adults excrete creatinine from 1-1.8 mg/day. Under heavy meat diet, wasting disease, prolonged starvation, fever *etc.*, it increases.

**Requirements:**

1. Creatinine standard solution: Dissolve 100 mg creatinine in 100 ml of 0.1 N HCl. Working standard solution may be prepared by appropriate dilution of the stock solution.
2. Saturated picric acid solution (about 1%).
3. Sodium hydroxide solution – 0.75 N.
4. Hydrochloric acid solution – 0.1N.
5. Sodium tungstate: 10%
6. Sulfuric acid - 2/3 N

**Preparation of protein free filtrate:** To 1 ml urine/blood sample, add 8 ml distilled water, 0.5 ml of 2/3 N sulfuric acid and 0.5 ml of 10% sodium tungstate solution in a stoppered centrifuge tube and mix the contents. Then centrifuge at 3000 rpm for 10 min and collect the supernatant.

**Procedure:**

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 1.5 ml of saturated picric acid solution and 1.5 ml of 0.75 N NaOH. Mix the contents of the tubes and incubate at room temperature for 15 min.
5. Then read the absorbance at 460 nm against blank.
6. Then plot the standard curve by taking concentration of creatinine along X-axis and absorbance at 460 nm along Y-axis.
7. From this standard curve calculate the concentration of creatinine in the given sample.

**Result:** The given unknown sample contains ----µg /ml.

<table>
<thead>
<tr>
<th>Volume of standard creatinine (ml)</th>
<th>Volume of distilled water (ml)</th>
<th>Concentration of creatinine (µg)</th>
<th>Volume of picric acid (ml)</th>
<th>Volume of NaOH (ml)</th>
<th>Incubate At Room Temperature for 10 Min</th>
<th>A460</th>
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<tbody>
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