Estimation of Uric Acid

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Aim: To estimate the amount of uric acid.

Principle: The uric acid reduces phosphotungstic acid in the presence of sodium carbonate to blue coloured complex. The concentration of uric acid is directly proportional to intensity of colour, which can be read at 700 nm.

Requirements:

1. **Tungstic acid**: Mix 25 ml of 10% sodium tungstate, 25 ml of 2/3 N H_2SO_4 and drop of phosphoric acid, make up to 400 ml with distilled water. Store in a brown bottle.
2. **Phosphotungstic acid (stock) solution:** Dissolve 50 g of sodium tungstate in about 400 ml of water. Add 40 ml of 85% phosphoric acid and reflux gently for 2 hours. Cool, transfer to 500 ml flask and make up to the mark with water. Keep this in a brown bottle
3. **Dilute solution for use:** Dilute 10 ml of the stock solution to 100 ml with water and store in a brown bottle.
4. **Sodium carbonate solution (10%).**
5. **Uric acid standard solution:** Dissolve 60 mg of lithium carbonate in 15-20 ml of water in a test tube. Heat the solution to 60 °C and pour on to 100 mg of uric acid taken in a small beaker. Stir until dissolved, heat further if necessary. Add 2 ml of 40% formalin and then slowly with shaking add 1 ml of 50% acetic acid. Make up to volume 100 ml and store in brown bottle.
6. **Uric acid working standard:** Dilute 1 ml of the stock to 200 ml with water. Store in a brown bottle. This contains 0.005 mg uric acid per ml.

Preparation of protein free filtrate: To 1 ml serum sample, add 9 ml of dilute tugstic acid in a stoppered centrifuge tube and mix the contents. Then centrifuge at 3000 rpm for 10 min and collect the supernatant as sample. 5 ml of supernatant is equivalent to 0.5 ml of serum.

Clinical Significance: The normal range of uric acid is 3-9 mg /100 ml of serum. Increased level indicates the diseases of joints.

Procedure:

1. Pipette out 0.0, 1, 2, 3, 4 and 5 ml of working uric acid standard in to the series of labeled test tubes.
2. Pipette out 5 ml of the given sample/ protein free filtrate in another test tube.
3. Make up the volume to 5 ml in all the test tubes. A tube with 5 ml of distilled water serves as the blank.
4. Now add 1 ml of sodium carbonate solution and 1 ml of dilute phosphotungstic acid solution to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and incubate for 3 min at 25 °C in a water bath.
6. Then record the absorbance at 700 nm against blank.
7. Then plot the standard curve by taking concentration of uric acid along X-axis and absorbance at 700 nm along Y-axis.
8. Then from this standard curve calculate the concentration of uric acid in the given sample.

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

Observations and Calculations

Volume of standard Uric acid (ml)	Volume of distilled water (ml)	Concentration of uric acid (µg)	Volume of Sodium carbonate solution (ml)	Volume of Phosphotungstic acid solution (ml)	A_{700}
0.0	5	00	1	1	0.00
1	4	5	1	1	
2	3	10	1	1	
3	2	15	1	1	
4	1	20	1	1	
5	0.0	25	1	1	
5 Unknown Sample	0.0	To be estimated	1	1	
