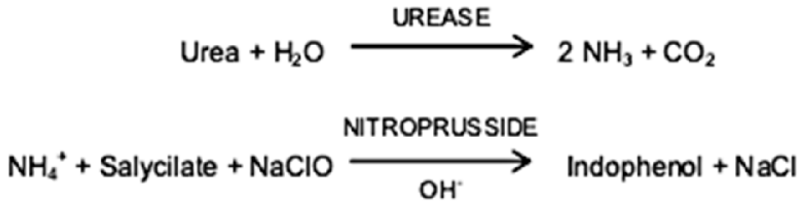
Experiment no.: 3

Experiment name: Determination of urea in the blood serum.

The aim of the Experiment:

Determination of urea in the blood using Urease/Salycilate *Enzymatic colorimetric method* ENDPOINT.

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green cromophore. The intensity of the color formed is proportional to the concentration of urea in the sample.



Equipment and martials used in the Experiment:

- − Photometer or colorimeter capable of measuring absorbances
- _ at600±10nm.
  _

  Constant temperature incubator set at 37 ºC.

- − Cuvettes with 1-cm pathlength.
- − Pipettes to measure reagent and samples.

- **REAGENT COMPOSITION**
  R1 Enzyme reagent. Urease > 500 U/mL. Stabilizers.
- **R2 Buffered chromogen**. Phosphate buffer 20 mmol/L pH 6.9, EDTA 2 mmol/L, sodium salycilate 60 mmol/L, sodium nitroprusside 3.4 mmol/L.
- **R3 Alkaline hypochlorite**. Sodium hypochlorite 10 mmol/L, NaOH 150 mmol/L.
- **Urea standard.** Urea 50 mg/dL (8.3 mmol/L)
  Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 909b.

- **Working reagent**. Mix 1 volume of **R1** + 24 volumes of **R2**. Stable
- for 4 weeks at 2-8 ºC and for 7 days at 15-25 ºC.

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. **PROCEDURE**
    1. Bring reagents and samples to room temperature.
    2. Pipette into a cuvette:

TUBES	Blank	Sample	CAL.Standard
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
CAL.Standard	–	–	10 µL

3. Mix and incubate for 5 minutes at 37 ºC or for 10 minutes at room temperature (16-25 ºC).

4. Pipette:

R3	1.0 mL	1.0 mL	1.0 mL

5. Mix thoroughly and incubate the tubes for 5 minutes at 37oC or for 10 minutes at room temperature (16-25 ºC).

5. Read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank.

The color is stable for at least 2 hours protected from light.

Experiment data and results:

Serum, plasma **REFERENCE VALUES** [5]	
Newborns (< 10 days)	6.4 - 53.5 mg/dL (1.1 - 9.0 mmol/L)
Adults (12-60 years)	15 - 40 mg/dL (2.5 - 6.6 mmol/L)
In adults over 60 years of age, the reference interval is 17-50 mg/dL (2.8-8.3 mmol/L) and the concentrations tend to be slightly higher in males than in females. Urine	
Adults (normal diet)	26 - 43 g/24-h (428 - 714 mmol/24-h)
A high-protein diet causes significant increases in plasma urea concentrations and urinary excretion. It is recommended that each laboratory establishes its own reference range.	

CALCULATIONS

Serum, plasma

$A_{Sample} / A_{Standard} \times C_{Standard}$ = mg/dL urea

Samples with concentrations higher than 300 mg/dL (50 mmol/L) should be diluted 1:5 with saline and assayed again. Multiply the results by 5.

Conclusion:

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