

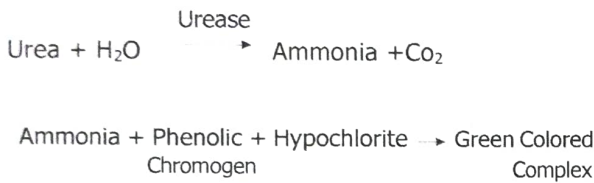
**Method:**

Urease - Berthelot Method.

**Clinical Significance:**

Urea is the end product of the protein metabolism. It is synthesized in the liver from ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy.

**Test Principle:**



Intensity of the color formed is directly proportional to the amount of urea present in the sample.

**Sample material:**

Serum, Plasma or urine. Diluent Urine 1 + 49 distilled or deionized water before the assay (Result X 50). Stability in serum/plasma 7 days at 2-8°C and Stability in Urine 7 days at 2-8°C.

**Reagents in the pack / kit:**

- R1 : Chromogen Reagent
- R2 : Enzyme Reagent
- R3 : Buffer Reagent
- Standard : Urea standard 40mg/dL

**Initial concentrations of solutions:**

**Reagent**

Urease	30,000U/L
Sodium Hypochlorite	10mmol/L
Sodium Hydroxide	400mmol/L
Sodium Salicylate	40mmol/L
Activator & Stabilizers	

**Standard**

Urea Standard 40mg/dL

**Normal Reference Range:**

Serum, Plasma 14 - 40mg/dL  
Urine Upto 20g/L

Each Laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

**Caution:**

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. Always use safety pipettes.

**Preparation and Stability of Solutions: Working Reagent**

Unopened reagent and standard are stable till the expiry date stated on the label when stored at 2-8°C.

**Note:** Please do not mix R1 & R2 prior to testing

Parameters	
Reaction Type	End Point (Increasing)
Wavelength	578nm (570 – 620nm)
Light Path	1cm
Reaction Temperature	37 <sup>0</sup> C
Blank/ Zero Setting	Reagent
Reagent Volume	1000µL
Sample volume	5µL
Incubation Time	8mins
Standard Concentration	40mg/dL
Low Normal	14mg/dL
High Normal	40mg/dL
Linearity	300mg/dL

**Procedure:**

Wavelength

: 570nm (Hg 578nm) Yellow

Cuvette

: 1cm light path.

Addition Sequence	Blank	Standard	Test
Reagent R1	500µL		
Reagent R2	25µL		
Standard	-----	5µL	-----
Specimen	-----	-----	5µL
Mix and incubate for 3 minutes at 37°C (5 mins.at R.T.)			
Buffer Reagent (R3)	500µL	500µL	500µL
Mix and incubate for 5min.at 37°C (10 min at R.T.)			



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Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against Blank (B), at 578nm(570-620nm). The final color is stable for 10 hrs at R.T.

**Calculation:**

$$\text{Urea (mg/dL)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Concentration of standard (mg/dL)}$$

$$\text{BUN (mg/dL)} = \text{Urea (mg/dL)} \times 0.467$$

**Product Features:**

Liquid stable Two Reagent system. Highest

Linearity 300mg/dL.

Urea & BUN value can be calculated.

No interference from Hemoglobin and Bilirubin. Suitable for colorimeter and Analyzers.

Results comparable with other enzymatic methods like Urea (GLDH).

**Linearity:**

The procedure is linear upto 300mg/dL. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

**Quality Control:**

Use of clean glassware, accuracy of pipetting and proper reaction temperature control are factors which can affect the results.

Use of commercial reference control serum is recommended for checking the proper functioning of instrument and reagent.

**Note:**

Enzyme reagent (R2) may appear slightly hazy, but after mixing it with buffer reagent (R3) its haziness disappears and this does not affect the performance of the kit.

Any contamination by ammonia or ammonium salts lead to erroneous results, hence plasma should not be collected with Fluoride or Heparin Ammonium salts.

The working (R1+R2) reagent is not stable at elevated temperatures and should be stored back at 2-8°C immediately after use.

The chromogen reagent (R1) contains chlorine. The bottles should be opened only when required and closed tightly after use to prevent the loss of active chlorine.

Pipettes and glassware must be free of all chemical contamination.

Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.

Before the assay bring all the reagents to the room temperature.

Avoid contamination of the reagent during assay process.

**References:**

1. Berthelot, M.P.E. (1859) Report chim.Appl.2884
2. Fawcett, J.K.Scott, J.E.(1960) J.Chim.Pathol.13:156