Hemezyme
GENUINE QUALITY, RELIABLE RESULTS

# UREA BERTHELOT

(UREASE – BERTHELOT METHOD)

**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:**

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + Co_2$$

$$\text{Ammonia} + \underset{\text{Chromogen}}{\text{Phenolic} + \text{Hypochlorite}} \rightarrow \underset{\text{Complex}}{\text{Green Colored}}$$

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 | $37^0C$ |
| 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 for10 hrs at R.T.

**Calculation:**

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

BUN (mg/dL) = Urea(mg/dL) X 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