UREA BERTHELOT
(BERTHELOT Method)

DIATEK

CLINICAL SIGNIFICANCE :

Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is metabolized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body. The test is frequently used for the differential diagnosis of prerenal hyperuremia (cardiac decompensation, water depletion increased protein catabolism), renal hyperuremia (glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, tubular necrosis) and postrenal hyperuremia (obstructions of the urinary tract).

TEST PRINCIPLE :

$$\text{UREA} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_4^+ + \text{Salycilate} \xrightarrow[\text{Sodium Hypochlorite}]{\text{Nitroprusside}} \text{Indophenol} + \text{NaCl}$$

Urea in the specimen is converted to ammonium and carbon dioxide by urease, and then the product ammonium reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitropruside as coupling agent to yield green colored complex. The intensity of the color formed is proportional to the concentration of urea in the sample.

REAGENT COMPOSITION

R1a: Urea Enzyme Concentrate (Urease >200kU/L)
R1b: Urea Buffer
Phosphate-Buffer (pH 7.3) - 50 mmol/L, Na Salicylate - 400 mmol/L, Na Nitroprussiate 10 mmol/L

R2 : Urea Color Reagent
Phosphate-Buffer 50 mmol/L, Hypochlorite : 50 mmol/L
Sodium Hydroxide - 50 mmol/L

Urea Standard : 40 mg/dL

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date.

KIT CONTENTS

CODE No.	*URB01*	*URB02*	*URB03*
Pack size :	(2x50 ml)	(2x100 ml)	(4x100 ml)
Reagent 1a	1 x 0.75 ml	1 x 1.5 ml	2 x 1.5 ml
Reagent 1b	1 x 50 ml	1 x 100 ml	2 x 100 ml
Reagent 2	1 x 50 ml	1 x 100 ml	2 x 100 ml
Standard (40mg/dL)	1 x 2 ml	1 x 2 ml	2 x 2 ml

REAGENT PREPARATION

Working Reagent (R1) is prepared by transferring Reagent 1a in the bottle of Reagent 1b. Use fresh tips and rinse the Reagent 1a bottle with the solution of 1b and ensure complete transfer. Appearance of Reagent 1a is slightly hazy / turbid. Haziness / Turbidity disappears once added to Reagent 1b. The working reagent is stable upto 4 months at 2 - 8 ºC. Affix the Working Reagent (R1) sticker on the bottle after Reconstitution is complete. Avoid keeping the reconstituted reagent at Room Temperature for a long time

INTERFERENCES

Gross haemolysed and lipemic sample should not be used The following analytes were tested up to the levels indicated and found not to be interfered by Hb upto 200 mg/dl, and Total Bilirubin upto 10 mg/dl.

SAMPLES : Serum or Heparinised / EDTA Plasma (Do not use Ammonium salt and Sodium Fluoride as anticoagulants)

Serum should be separated from blood as soon as possible.

Urine sample to be diluted 1:100 with normal saline and the final result should be multiplied with dilution factor 100.

ASSAY CONDITIONS:

Wavelength : ..... 578 nm
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction (Mode)..... End Point
Standard Concentration..... 40 mg/dl
Linearity..... 300 mg/dl
Unit..... mg/dl
Reaction Slope..... Increasing
Blanking..... Reagent

ASSAY PROCEDURE :

	B	S	T
Working Reagent (R1)	1000 l	1000 l	1000 l
Urea Standard		10 l	
Specimen			10 l
Mix and incubate for 5 minutes at 37°C (10 mints at RT)			
Reagent (2)	1000 l	1000 l	1000 l

Mix and incubate for 5 minutes at 37°C (10 mints at RT)
Mix and read absorbance of Standard (S) and Test (T) against Blank (B) at 578nm (570 - 620nm). Final color is stable upto 1hr.

CALCULATIONS :

$$\text{Urea (mg/dL)} = \frac{\text{Abs of Test}}{\text{Abs of Standard}} \times 40$$

Blood Urea Ntrogen (BUN) in mg/dL = Urea (mg/dL) x 0.467

REFERENCE RANGE :

Serum: 10 - 50mg/dl Urine: 15-35 gm/24h

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Use clean glassware and microtips while pipetting Reagents. **The reaction is highly sensitive to ammonium ion hence the reagent must be kept and test performed in ammonia free zone.**
3. Avoid contamination of the reagent during the assay process.
4. Before the assay begins, bring all the reagents to room temperature.
5. **Reagent Blank is pale yellow color and Working Reagent performance its best till the Blank absorbance reach upto 0.25 against Distilled water at 578 nm during storage.**
6. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.
7. Programmes for specific autoanalysers are available on request.
8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear upto Urea Concentration 300 mg/dl.
The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 4 with Normal Saline and multiply the result by 4.

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