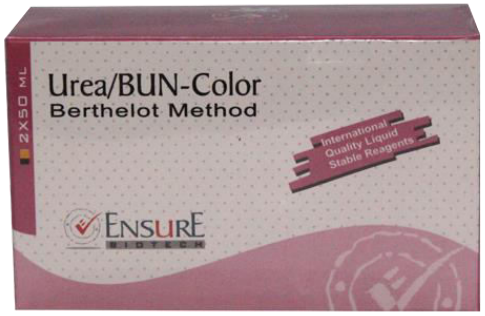
UREA BERTHELOT



PRINCIPLE

Urea breaks down Urea into Ammonia and Carbon dioxide. in alkaline medium ammonia reacts with Hypochlorite and Salicylate to form a dicorboxy indophenol is a colored compound. The reaction is catalyzed by sodium nitropresside. The intensity of the color is directly proportional to the Urea present in the serum, which is measured at 578 nm or with yellow filter

Urea + H_20 → Ammonia + Co_2

Ammonia + Hypochlorite +Salicylate → 2 Di-Corboxy indo phenol +Nacl (Green color)

CLINICAL SIGNIFICANCE

The determination of Urea is the most widely used test for the evaluation of kidney function. The test is frequently in conjunction with the determination of creatinine for the differential diagnosis prerenal hyperurenmia, renal hyperurenmia , and post renal hyperurenmia.

Urea is the final degradation product of protein and ammonia acid metabolism. It constitutes the largest function of the non -protein nitrogen compound in the blood. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver. The circulating level of urea depends upon protein intake, protein metabolism and kidney function. This is the most important catabolic path way for eliminating excess nitrogen in the human body.

Elevated serum urea levels may be due to pre -renal or postrenal etiologies. Pre -renal causes could be cardiac related or due to increase protein catabolism . renal cases include glomerulonephritis, chronic nephritis, nephritis syndromes and other kidney diseases. Post renal cases include observation of the urinary tract . The reduce level are found in malnutrition, hepatic failure and pregnancy.

Ensure Urea incorporates liquid reagent for estimation of urea photometrically by the berthlot method.

SPECIMEN COLLECTION

Serum! Heparinized or EDTA plasma (Do not use Ammonium salts and sodium Fluoride as anticoagulants) Urine (dilute 1:100 with distilled water and multiply the result with dilution factor)

PRECAUTIONS

1. The reaction IS highly sensitive to ammonium salts enough care should be taken to keep glass ware compulsory clean and to run the assay in an ammonia free atmosphere.
2. Urease reagent pale yellow in colored due to which the blank absorbance reads around 0.200 Abs at 578 nm against distilled water. The absorbance of standard and test read against reagent blank at 578 nm.nulifies the absorbance of urease reagent.
3. The test is not influenced by hemoglobin up to 200mg / dl, and by Bilirubin up to 10mg/dl.

KIT CONTENTS &STORAGE (2 x 50 ml) (4 x 50 ml)

Reagent-i Urease reagent	1x50 ml	2x 50 ml
Reagent-iA Enzyme concentrate	1 Vail	2 Vail
Reagent-Z Alkaline Buffer	1x50ml	2x50ml
Reagent3 Standard (40 mg/ dl)	2ml	2ml

All the reagents must be storage at 2-8°C and are stable till the expiry mentioned on the labels

REAGENT PREPARATION :

1. Transfer the entire Enzyme Concentrate (1A) into Urease Reagent (t) with the dropper (or) micro tip provided.
2. Once the enzyme concentrate is transferred. rinse the enzyme concentrate Valle with little urease reagent and transfer the residual enzyme to ensure better reconstitution.
3. The reconstituted stable for 4 months when proper storage conditions are strictly mentioned.
4. Avoid keeping the reconstituted reagent at room temperature for long time.
5. It is advised to keep the reagent back at 2-8‘Conce the assays is over.
6. Slight haziness! turbid in the enzyme concentrate Vaile disappears once added to urease reagent and does not affect test performance and result

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SYSTEM PARAMETERS:

Reaction Type (Mode)	: End Point
Wave Length	: 578 nm (570-620)
Flow Cell temperature	: 37°C
Blank	: Reagent
Units	: 40
Standard Conc	: 2000 pt
	(R1 -1000µl.R21000µ)
Reagent Volume	:10 µl
Sample Volume	: 10 µl
Incubation	: 10min
Low Normal	: 10
High Normal	: 50

PROCEDURE

Pipette in to test tubes labeled Blank(B), Standard(S), and Test(T) as follows

WorkingReagent	1.0ml	1.0ml	1.0ml
Standard(40mg/dl	-----	10 µl	----
specimen	-----	-----	10 µl
Mix and incubate for 5 minutes at 370 C(10 minutes at R.T.)			
Buffer R2	1.0ml	1.0ml	1.0ml
Mix and incubate for 5 minutes at 37^0C(10 minutes at R.T.)			

Mix and read absorbance of Standard (8) and Test (T) against Blank (B) at 578 nm or with yellow filter.

The final color is stable for 30 mints at RT.

CALCULATIONS

a) Serum / Plasma Urea in mg/dl = (Abs of T/Abs of S) x 40 mg / dl

b) Blood Urea Nitrogen in mg/dl = a x 0.487

c) Urine Urea in gm / 24 hrs = a x 24 hrs urine Vol In Ltrs.

LINEARITY

ENSURE UREA kit is Linear up to 300 mg/dl. Sample above this concentration should be diluted 1:9 with Saline and the result multiplied by 10.

NORMAL RANGE:

Serum/plasmaUrea	:	10-50 mg/dl.
Urine Urea	:	25-43gm/24hrs
Serum /Plasma Urea Nitrogen	:	523mg / dl

It is recommended mat laboratories establish their own Normal range.

BIBLIOGRAPHY :

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