Urea - EGD

(Berthelot Method)

Code: 11037 / 11038 (2x50ml/2x100ml)

(For the analyser/colorimetric estimation of UREA in Serum, Plasma/Urine)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Elevated serum levels may be due to pre-renal, or post-renal etiologies. Pre-renal causes could be cardiac related or due to increase protein catabolism, Renal causes include glomerulonephritis, chronic nephritis, nephritic syndromes and other kidney diseases; post-renal causes include obstruction of the urinary tract.

Liquichem UREA incorporates liquid reagents for estimation of urea photometrically by the Berthelot Method. This method offers a high degree of precision and specificity due to Urease and high sensitivity due to highmolar absorption of the final color.

PRINCIPLE:

Urease catalyses the conversion of Urea to Ammonia and Carbondioxide. The Ammonia released reacts with a mixture of salicylate, hypochlorite and Nitroprusside to yield a blue-green colored compound (Indophenol). The intensity of color produced is proportional to the concentration of urea in the Sample and is measured photometrically at 570nm or with yellow filter.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

$$NH_3 + Salicylate \xrightarrow[Hypochlorite]{Nitroprusside} 2\text{-}2\text{-Dicarboxy Indophenol}$$

REAGENTS:

	2x50 ml	2x100 ml
1. Urease Reagent	1x50 ml	1x100 ml
2. Enzyme Concentrate	1 Vial	1 Vial
3. Color Reagent	1x50 ml	1x100 ml
4. Urea Standard (40 mg%)	1x2 ml	1x2ml

The reagents are ready to use and usable to the expiration date when stored at 2 - 8°C & Protected from light, if contamination is avoided.

WORKING REAGENT PREPARATION :

Transfer the entire Enzyme Concentrate(2) into Urease Reagent (1) with dropper or microtip, rinse the Enzyme Concentrate Vial with little Urease Reagent and transfer the residual enzyme to ensure better reconstitution. This reagent is stable for 4 months at 2-8° C.

SAMPLE:

Serum

Heparin or EDTA plasma (do not use ammonium salts and sodium fluoride as anticoagulants).

Urine (dilute 1: 100 with distilled water)

EXPECTED RANGE:

Serum/Plasma Urea : 10 - 50 mg%
Urine Urea : 25 - 43 gm/24 hrs.

LINEARITY:

Urea kit is linear upto 300 mg%

INSTRUCTIONS :

1. Urease Reagent is pale yellow in color due to which the Blank absorbance reads around 0.200 at 570 nm against distilled water. The absorbance of Standard and Test read against Reagent Blank at 570 nm nullifies the absorbance of urease Reagent.
2. Avoid keeping reconstituted reagent at room temperature for a long time.
3. Slight haziness / turbidity in the Enzyme concentrate vial disappears once added to Urease Reagent and does not affect test performance and results.

DIRECTIONS FOR USE ON ANALYSERS :

Reaction Type	:	End point with Std.
Wave Length	:	570nm (Yellow Filter)
Incubation Temp	:	37°C
Incubation Time	:	10 min.
Standard	:	40 mg%
Linearity	:	300 mg%
Unit	:	mg%

PROCEDURE :

Pipette in a clean, dry test tubes labelled Blank (B), Standard (S) and Test (T) as follows

	B	S	T
Urease Reagent (ml) (working)	1.0	1.0	1.0
Urea Standard (ml)	-	0.01	-
Specimen (ml)	-	-	0.01
Incubate for 5min. at 37°C/10 Min at RT			
Colour reagent (ml)	1.0	1. 0	1. 0

Mix and incubate for 5 min at 37°C/10 min. at RT & read absorbance of standard (S) and Test (T) against Blank (B) at 570nm or with yellow filter. The final color is stable for 30 min at R.T.

CALCULATIONS :

a) Serum / Plasma

$$\text{Urea in mg\%} = \frac{\text{Abs. of (T)}}{\text{Abs. of (S)}} \times 40 \text{ (Std. Conc)}$$

b) Blood Urea Nitrogen in mg% = a x 0.467

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

NOTES :

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

Chaney, A.L. and Marbach, E.P. (1962) Clin. Chem. 8., 130

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