

Urea

DAM Method

Code : 10022 (2 x 100 ml)

(For the Colorimetric estimation of Urea in Plasma / Serum and urine)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Urea is a major component of Non-Protein Nitrogen (NPN) in Serum. It is an end product of protein catabolism and is formed mainly in liver and excreted by Kidney. Urea estimation is mainly used for the diagnosis of renal disease (Urine urea estimation is of use, only as a part of urea clearance test).

For Urea assay the popular methods are based on urease Nesslerization, Berthelot reaction and the condensation with Diacetyl monoxime (DAM). UREA KIT is based on the condensation of Urea with DAM in an acidic medium. The presence of ammonia does not interfere in this method. The method is one step, rapid and very simple, and does not need deproteinization of specimen. The reagents are modified and stabilized to give accurate results throughout the shelf life.

PRINCIPLE:

Urea reacts with DAM in an acidic medium to produce a colored complex. The color is intensified by using thiosemicarbazide and cadmium salt. The intensity of the color produced is proportional to Urea concentration.

REAGENTS:

1. Urea Reagent 100 ml
2. DAM Reagent 100 ml
3. Standard (30 mg%) 3ml

The reagents are stable at 2 - 8°C till the expire date mentioned on the label.

SAMPLE :

Serum / Plasma or Urine

Urine should be of 24 hours collection. Dilute Urine specimen 1:20 using distilled water of deionised water before use.

EXPECTED RANGE:

Serum/Plasma Urea : 14 - 40 mg%
Urine Urea : upto 20 gm/lit.

LINEARITY :

This method is linear upto 70 mg%. Samples exceeding 70 mg% should be dilute and reassayed. The result has to be multiplied by the dilution factor.

INSTRUCTIONS :

1. Urea Reagent is corrosive. Avoid contact with skin and eyes.
2. Use glassware cleaned with chromic acid then dried.

PROCEDURE :

Pipette into clean, dry tubes labelled Blank (B) Standard (S) and Test (T) and add the reagents in the following order.

	B	S	T
Deionised Water (ml)	4.0	4.0	4.0
Urea Reagent (ml)	1.0	1.0	1.0
DAM Reagent (ml)	1.0	1.0	1.0
Standard (ml)	—	0.02	—
Serum/Plasma/dilute	—	—	0.02
Urine (ml)			

Mix well after each addition and keep the test tubes in a water bath at 100°C for exactly 10 minutes. Cool under running tap water and then measure the absorbance of Standard (s) and Test (T) against Blank (B) on photocolormeter using green filter or on spectrophotometer at 520 nm within 30 minutes.

CALCULATIONS :

$$\text{Urea Concentration mg\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 30 \text{ (Std. Conc)}$$

$$\text{Urea Nitrogen mg\%} = \text{Urea (mg\%)} \times 0.467$$

$$\text{Urine Urea in gm / litre} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 6$$

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:

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3. Wybenga, D.R. (1971) Clin. Chem. 17:891.

Manufactured in India by :

M/s Excel Diagnostics Pvt. Ltd.

Plot NO. 89, Road No.8, ALEAP I.E., Near Pragathi Nagar,
Opp. Kukatpally JNTU, Hyderabad - 500 090 (A.P.) INDIA.
E-mail : edpl@rediffmail.com Visit us at - www.exceldiag.com