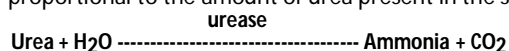


TECHNICAL SUMMARY:

Urea is the main product of the protein metabolism. It is synthesised in the liver from the 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 disease, urinary obstructions, shock, congestive heart failure & burns. Decreased levels are found in liver failure & pregnancy.

PRINCIPLE:

UREA hydrolyses by an enzyme urease to ammonia & CO₂. Ammonia formed is further reacts with phenolic chromogen & Hypochlorite to form a green Coloured complex. The intensity of the colour formed is directly proportional to the amount of urea present in the sample.



Ammonia + Phenolic chromogen + Hypochlorite ----- Green Coloured complex.

STORAGE & STABILITY:

All the reagents are stable at 2-8°C. upto the expiry date mentioned on the label.

SAMPLES:

Serum or Plasma or Urine

REFERENCE VALUES:

Serum / plasma: 10 - 45 mg / dl.
 Urine : upto 20 gm / L.

{Dilute urine sample with 1+49 with DI water & multiply the results by 50.}

[It is recommended that each laboratory should establish its own normal range]

REAGENT PREPARATION:

For 2 x 50 ml kit:

Mix One Vial of R₁ - Buffer reagent (50 ml) with 2.5 ml of R₂ - Enzyme Conc. reagent. The reconstitution stability of working reagent is 30 days at 2-8°C. or one can follow the mixing of R₁ & R₂ as per the requirement of assay procedure.

For 2 x 100 ml kit:

Mix One Vial of R₁ - Buffer reagent (100 ml) with 05 ml of R₂ - Enzyme Conc. reagent. The reconstitution stability of working reagent is 30 days at 2 - 8°C. or one can follow the mixing of R₁ & R₂ as per the requirement of assay procedure.

ASSAY PROCEDURE:

Wavelength / Filter : 578 nm (570 - 600 nm)
 Temperature : 37°C
 Cuvette : 1 cm light path.

	B	S	T
R ₁ -Buffer Reagent	1000ul	1000ul	1000ul
R ₂ - Urease Conc. Reagent	50 ul	50 ul	50ul
S ₁ -Urea Standard(40 mg/dl)	-----	10 ul	-----
Serum	-----	-----	10 ul
R ₃ -Chromogen Solution	1000ul	1000ul	1000ul

Mix well and incubate for **03 minutes** at 37°C or 5 minutes at R.T. & then add.

Mix well & incubate for **05 minutes** at **37°C** or **10 minutes** at **R.T.** and measure the absorbance of the Standard (**Abs.S**) and Test Sample (**Abs.T**) against the reagent blank .

CALCULATION:

$$\text{UREA (mg /dl)} = \frac{(\text{Abs. Test}) - (\text{Abs. B})}{(\text{Abs. Std.}) - (\text{Abs. B})} \times 40$$

LINEARITY:

The reagent is linear upto **250 mg/dl**

If values exceed this limit, dilute the sample with DI water & repeat the assay. Multiply the result with proper dilution factor.

ASSAY PARAMETERS:

- Mode of reaction : End point
- Slope of reaction : Increasing
- Wavelength : 578 nm (570 - 600 nm)
- Temperature : 37°C
- Blanking : Reagent blank
- Standard Conc. : 40 mg/dl
- Reaction time : 03 min + 05 min at 37°C
- Reagent Volume : 1000 ul + 1000 ul
- Sample Volume : 10 ul
- Linearity : 250 mg / dl

REFERENCES:

Berthelot, M.P.E., (1859) REPORT CHIM. APPL. 2884.

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