UREA-UV (GLDH KIT)

PRINCIPLE:

The enzymatic reaction sequence employed in the assay of Urea UV is as follows:

$$\text{UREA} + H_2O \xrightarrow{\text{UREASE}} 2NH_4^+ + 2HCO_3$$

$$\text{2 - OXOGLUTARATE} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L - GLUTAMATE} + NAD^+ + H_2O$$

REAGENTS & STABILITY:

01. Reagent R1 : Ready - To - Use
02. Reagent R2 : Ready - To - Use
03. Urea Standard : Ready - To - Use

Avoid Contamination of Ready-To-Use Reagents. Always use fresh pipette tips. Keep always the caps tightly closed.

The above Reagents R1 & R2 are ready – To – Use and are stable until expiry dates mentioned on the label.

REAGENT PREPARATION:

Mix 4 parts of R1 with 1 part of R2. The combined Reagent is stable for 4 weeks at 2^0 - 8^0 C.

COLLECTION AND HANDLING OF SPECIMEN:

1. Serum is the sample of choice.
2. Fresh Urine can also be used. Dilute 1 ml Urine with 100 ml of distilled water.

NOTE:

1. This method is optimised for 2 – point Kinetic measurement. It is strictly recommended to perform the test on semiautomatic and automatic instruments.
2. Bilirubin upto 40 mg/dl, Haemoglobin upto 200 mg/dl, ascorbate up to 30 mg/dl and Triglycerides up to 2000 mg/dl do not interfere. Ammonia interferes, hence do not use Ammonium Heparinate for collection of plasma.
3. To convert Urea into Bun multiply by 0.47 to convert Bun into Urea multiply by 2.14. The reagents contain Sodium Azide as preservative. Do not swallow and avoid contact with skin and mucous membranes.

PROCEDURE:

Method	: Fixed Time
Wave Length	: 340 nm
Temperature	: 37^0 C
Cuvette	: 10 mm path length
Standard	: 50 mg/dl (refer the vial label)
Pre-Reaction Time	: 30 Sec
Reaction Time	: 60 Sec.
Slope	: Decreasing

Pipette into cuvettes	Macro	Semi-Micro
Reagent(R1+R2)	800+200 ul	400+100 ul
Sample/Standard	10 ul	5 ul

Mix well & then measure absorbance (A1). After exactly 60 Sec. measure absorbance A2. A=A1-A2. (fixed time chemistry) subtract A of reagent blank from A of sample/standard and use the result for the calculation.

CALCULATIONS:

$$\text{Urea mg/dl} = \frac{\text{A. SAMPLE}}{\text{A. STANDARD}} \times \text{Conc. of Standard.}$$

EXPECTED VALUES:

: Serum : 10-50 mg/dl
: Plasma: 10-50 mg/dl

LINEARITY : 300 mg/dl

REFERENCES:

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