UREA BUN

GLDH Method

Intended Use:
Urea test reagent/kits is a medical device intended for the estimation of urea/Blood urea nitrogen (BUN) in serum or plasma.

Clinical Significance

Urea is the chief end product of protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function. *Azotemia (* an abnormal increase in plasma urea level) is seen mainly in renal disorders, dehydration, increase protein catabolism, high-protein diets, or gastrointestinal hemorrhage. There are two types of azotemia. The first, *prerenalazotemia*, is caused by impaired perfusion of the Kidneys due to decreased cardiac output or for any of the former causes. The second, *postrenalazotemia*, is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinarytract.

The clinical significance of the urea level in plasma is usually determined in conjugation with the plasma creatinine level. In prerenal azotemia, an increase in the plasma urea level is usually associated with a normal plasma creatinine level, where as in postrenal azotemia, there is an increase in both the urea and the plasma creatinine levels. A decrease in the urea plasma level may be associated with acute dehydration, malnutrition, and pregnancy.

Principle

Urea is hydrolyzed by urease to ammonia and carbon dioxide. The ammonia is converted to glutamate by glutamate dehydrogenase (GlDH) in the presence of NADH and oxoglutarate.[1,2]

The reaction is monitored kinetically at 340 nm by the rate of decrease in absórbanse resulting from the oxidation of NADH to NAd[2], proportional to the concentration of urea present in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$\text{2-Oxoglutarate} + NH^+ + 2NADH \xrightarrow{\text{GLDH}} \text{Glutamate} + 2NAD^+ + 2H_2O$$

Reagent Composition:

R1 Enzyme Reagent	NADH 1.50 mmol/L.
R2 Urea BUN Reagent	TRIS buffer 125 mmol/L pH 7.4, α - Ketoglutarate 10 mmol/L, Urease> 140 U/ml, Glutamate dehydrogenase >120 U/ml
Standard	50mg/dl

Working Reagent Preparation
Reagent is ready to use.
For sample start assays a single reagent is required.
Mix 8ml of Urea BUN Reagent (R2) with 2ml of Urea Enzyme Reagent (R1).

The Working Reagent is stable for at least 30 days when stored at 2–8°C.

Stability and Storage
Store at 2-8ºC
All the kit contents are stable until the expiry date stated on the label. Do not use reagents beyond the expiration date. Store the vials tightly closed protected from light and prevent contaminations during the use.

Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.100 in 1cmcuvette.

Materials required
- Photometer or spectrophotometer with a thermostatted cell compartment set at 37ºC, capable of reading at 340nm.
- Stopwatch, strip-chart recorder orprinter.
- Cuvettes with 1-cmpathlength.
- Pipettes to measure reagent andsamples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis and urine (see Notes). Other anticoagulants (ammomiumheparinate of double oxalate of potassium and ammonium) must not be used.
Urea in serum, plasma or urine is stable 7 days at 2–8°C. Freeze for longer storage.

Known Interfering Substances
- Lipemia (intralipid< 5 g/L) does notinterfere.
- Bilirubin (40 mg/dL), hemoglobin (< 4 g/L), does not interfere.
- Contamination of glassware and Water by ammonia, will give falsely elevated results.
- Fluorides used commonly as anticoagulants inhibit the urease of the substrate[4].

Assay Procedure

Wavelength/ Filter	:	340 nm
Temperature	:	37°C
Light Path	:	1 cm

Pipette into clean dry test tube labeled as Standard (S) and Test (T)

Addition Sequence	Std (µl)	Test(µl)
Working Reagent(WR)	1000	1000
Standard(S)	10	-
Sample	-	10

Mix well and read the initial absorbance A1 for the standard and test after exactly 30 sec. Read another absorbance A2 of the Standard and Test exactly 60 sec later. Calculate the change in absorbance ΔA for both the standard and test

Calculation

$$\text{Urea in mg/dl} = \frac{\Delta \text{ Abs of Test}}{\Delta \text{ Abs of Standard}} \times 50$$

Quality Control

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Linearity

This procedure is linear upto 300 mg/dl. If values exceed this limit , dilute the sample with normal saline (NaCl 0.9%) and repeat assay. Calculate the value using the proper dilution factor.

Reference Values

Serum and Plasma : 14 - 40 mg/dl
Urine : 20 g/l

It is recommended that each laboratory establish its own mormal range representing its patient population.

General System Parameters

Mode	Fixed Time Kinetic
Reaction	Decreasing
Wavelength	340 nm
Blank with	Distilled Water
Sample Volume	10µl
Reagent Volume	800µl (R2) + 200µl (R1)
Std Conc.	50 mg/dl
Incubation Temp.	37^0C
Delay Time	30 sec
Measuring Time	60 sec
Linearity	Up to 300 mg/dl
Unit	mg/dl

Notes

Any contamination by ammonia or ammonium salts lead to erroneous results. Hence plasma should not be collected within fluoride or Heparin ammonium salts. The working enzyme reagent is not stable at elevated temperatures and should be stored back at 2-8°C immediately after use.

Reference

1.Fawcett J.K, Scott.J.E (1960) J.Chem.Pathol. 13:156

2.Chary.A.(1969) Clin.Chem 8:130

For *in vitro* Diagnostic use only