ANA BIO CLR UREA - GLDH

(GLDH Method)

Intended Use

Urea is a reagent kit used for the quantitative determination of Urea / **B**lood **U**rea **N**itrogen (BUN), based on enzymatic method using Urease and Glutamate dehydrogenase (GLDH) enzymes.

Principle

Urea is hydrolyzed to ammonia and carbon dioxide by urease. This Ammonia reacts with α-ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD^+ in this reaction, which is measured as decrease in absorbance at 340 nm. The rate of decrease in absorbance at 340 nm is directly proportional to BUN / Urea concentration in the specimen.

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

$$NH_3 + \alpha\text{-ketoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+$$

Kit presentation

1. Reagent R_1
2. Reagent R_2
3. Standard - Urea (40 mg/dl) : BUN (18.69 mg/dl)

Working Reagent Preparation

Prepare working reagent by mixing Reagent R_1 and Reagent R_2 in the ratio 4:1 as per the number of tests required.

Reagent storage and stability

The reagent kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label.
The working reagent (4 R_1 + 1 R_2) is stable for 30 days at 2° - 8°C.

Specimen collection and preservation

Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can also be used. Anticoagulants such as ammonium heparin and fluoride should not be used. Blood Urea Nitrogen (BUN) concentration in serum / plasma is stable for 6 days at 2° - 8°C and for a month when stored at -20°C. The samples should be brought to room temperature prior to use.

Assay guidelines for Analyzer

Reaction type	Fixed time (Two Points)
Reaction Slope	Decreasing
Wavelength	340 nm
Temperature	37°C
Delay time	30 seconds
Read time	90 Seconds
Blank	Distilled water
Sample volume	10 µl (0.01 ml)
Reagent volume	1000 µl (1.0 ml)
Urea / BUN Std. Con.	40 / 18.69 mg/dl
Low Normal (BUN / Urea)	5 / 10 mg/dl
High Normal (BUN / Urea)	21 / 45 mg/dl
Linearity (BUN / Urea)	Up to 250 / 530 mg/dl

Assay guidelines for Manual procedure

Prewarm the required amount of working reagent at 37°C before use. Perform the assay as given below:

Reagents	**Standard**	**Test**
Working Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	10 µl (0.01 ml)	-
Sample	-	10 l (0.01 ml)

1. Mix thoroughly and transfer the assay mixture immediately to the thermo stated cuvette and start the stop watch simultaneously.
2. Record the first reading at 30^{th} second and subsequently one more readings with 90^{th} seconds interval at 340 nm.

Calculation

1. Urea con. in sample (mg/dl) = $\frac{\Delta \text{ Absorbance of sample}}{\Delta \text{ Absorbance of standard}} \times 40$

2. BUN concentration (mg/dl) = $\frac{\Delta \text{ Absorbance of sample}}{\Delta \text{ Absorbance of standard}} \times 18.69$

Normal Range

Guidance value (Urea) : 10 - 45 mg/dl
Urea nitrogen : 5 - 21 mg/dl

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitations

- Fluoride should not be used as a anticoagulant as it inhibits urease activity and anticoagulants having ammonium ions should not be used because of extreme sensitivity of the colour reaction to ammonia.
- Reaction is linear up to 250 / 530 mg/dl. For higher values, dilute sample with normal saline and perform the assay. Multiply final result by dilution factor to get the real value.
- The working reagent is considered unsatisfactory and should not be used if the absorbance is less than 0.800 at 340 nm. against distilled water.
- Do not use strongly haemolysed samples.

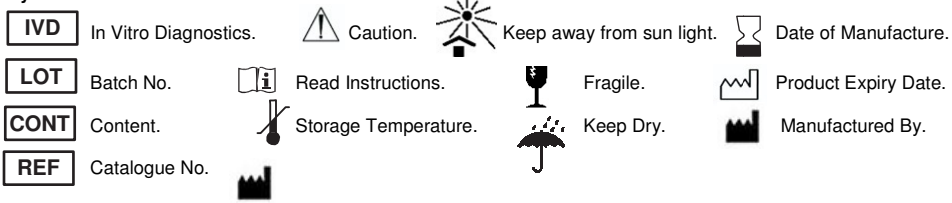
Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

References

1. Talke H., Schubert, G.E., Klin. Wochenschr, 43, 174, (1965).
2. Gutman, I., Bergemeyer, H.U., in "Methods of Enzymatic Analysis", H.U. Bergemeyer Ed., Academic Press (1974), p. 1791.
3. Tiffany, T.O., et al, Clin. Chem., 18, 829, (1972).
4. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, Pa : W.B. Saunders Company, 1995 : 622 – 626.

Symbols



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