ANA BIO CLR UREA

(Berthelot Method)

Intended Use

Urea is a reagent kit used for the quantitative determination of urea/blood urea nitrogen based on enzymatic method using Urease enzyme.

Principle

Urease splits urea into ammonia and carbon dioxide. Ammonia released in this reaction reacts with hypochlorite and Phenolic chromogen, to produce green colour. The absorbance of this green colour at 578 nm (570 – 620 nm) is directly proportional to the concentration of urea in specimen.

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

$$NH_3 + \text{Hypochlorite} + \text{Chromogen} \longrightarrow \text{Green colour}$$

Kit presentation

1. Enzyme R_1 : Enzyme Reagent
2. Reagent R_2 : Chromogen solution
3. Reagent R_3 : Hypochlorite solution
4. Standard : Urea (40 mg/dl)

Working Reagent Preparation

Prepare working reagent by mixing Reagent R_1 and Reagent R_2 in the ratio 1:9 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. DO NOT FREEZE THE ENZYME SOLUTION. The Hypochlorite solution is stable for 6 months at 2°- 8°C after the first opening.

Specimen collection and preservation

Blood should be collected in a clean and dry container (free of NH_3). Plastic or siliconized container should be avoided as it may prolong clotting time. Following anticoagulant may be used for plasma separation.

- EDTA 2 mg/ml of blood
- CITRATE 6 mg/ml of blood
- OXALATE 3 mg/ml of blood
- HAPARIN 200 IU/ml of blood

Ammonium salts of anticoagulants and sodium fluoride should not be used as anticoagulants.
Urea in the specimen is stable for a week when stored at 2° - 8°C and for a month when stored at -20°C

Assay guidelines for Analyzer

Reaction type	End point with standard
Reaction slope	Increasing
Incubation time	3 + 5 minutes at 37°C
Wavelength	578 nm (570 - 620 nm)
Blank	Reagent Blank
Blank Absorbance limit	< 0.200 Abs.
Sample Volume	10 µl (0.01 ml)
Reagent Volume	1000 µl (1.0 ml) + 1000 µl (1.0 ml)
Urea Standard Concentration	40 mg/dl
Factor Calculation	40 mg/dl ÷ Absorbance of standard
Low Normal	10 mg/dl
High Normal	45 mg/dl
Linearity	Up to 350 mg/dl

Assay guidelines for Manual procedure

Bring all the reagents to room temperature before performing the assay.

Reagents	Blank	Standard	Sample
Reagent R1	100 µl (0.1 ml)	100 µl (0.1 ml)	100 µl (0.1 ml)
Reagent R2	900 µl (0.9 ml)	900 µl (0.9 ml)	900 µl (0.9 ml)
Standard	-	10 µl (0.01 ml)	-
Sample	-	-	10 µl (0.01ml)
Mix and incubate for 3 minutes at 37ºC.			
Reagent R3	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0ml)
Mix and incubate at 37ºC for 5 minutes.			

1. Read the absorbance against reagent blank at 578 nm (570 - 620 nm).
2. The final colour is stable for 2 hrs. if not exposed to direct light.

Note: Reagent to sample ratio can be altered proportionality without affecting the performance of assay.

Calculation

1. Urea con. in sample (mg/dl) = $\frac{\text{Sample OD.}}{\text{Std. OD}}$ x Con. of Std.
2. BUN con.in sample (mg/dl) = $\frac{\text{Sample OD.}}{\text{Std. OD}}$ x 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 as well as anticoagulants having ammonium ions should not be used because of Inhibit the enzyme activity and extreme sensitivity of the colour reaction to ammonia. Reaction is linear up to 350 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. During assay, blank develops a prominent yellow colour. Only if the absorbance of the same exceeds 0.200 at 578 nm against distilled water blank, the reagent should be considered unsatisfactory and should not be used. Detergents containing ammonium ions and strong oxidizing disinfectants (sodium hypochlorite) should not be used for washing glass wares.

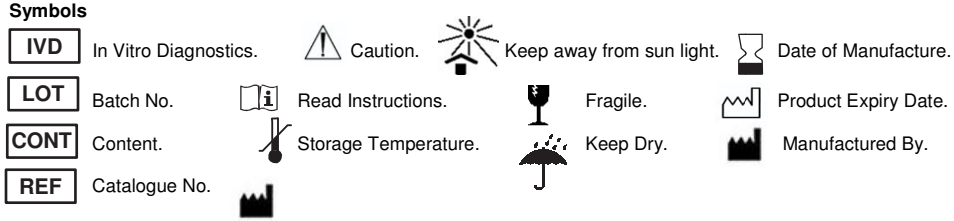
Quality Control

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, and cleanliness of glassware and accuracy of pipetting.

References

1. Webstar D., Clin. Chem. 23, 663 (1977).
2. Dumas B.T.; et al, Clin. Chem. Acta, 31, 87 (1971).
3. “Practical clinical biochemistry”, Harold Varley, V edition, Vol. I, pp 457 (1980).

Symbols



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