Urea Berthelot-LQ (Urease- Hypochlorite Method)

Invitro Diagnostic reagent kit for quantitative determination of Urea in serum, plasma or urine samples on Photometric System.

Reagent :
Reagent I : Buffer reagent
Reagent II : Enzyme reagent
Reagent III :Color developer(Hypochlorite solution)
standard : Urea Standard 50mg/dl(8.33 mmol/L)

Principle

The Berthelot reaction has long been used for the measurement of urea and ammonia. The present method is a modified Berthelot Method. The Urea colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonium by the use of urease. Ammonium ion then reacts with a mixture of salicylate, sodium nitroprusside and hypochlorite to yield a blue-green chromophore. The intensity of the color formed is proportional to the urea concentration in the sample.

CLINICAL SIGNIFICANCE

Urea is the final result of the metabolism of proteins; It is formed in the liver from their destruction. It can be elevated in blood in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Summary

Urea is waste product formed in the liver and filtered out by the kidneys. The increased concentrations of Urea are found in kidney problems, urinary tract obstructions, and congestive heart failures. Its decreased concentrations are observed during hepatic failures and also in pregnant women. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia.

Storage Instructions and Reagent Stability

When stored between 2-8°C All the reagents are stable until the expiration date stated on the bottle and kit box label.

Reagent Composition

Reagent:- 1 Phosphate buffer 20mmol/L,EDTA 2mmol/L,Sodium salycilate 60mmol/L,sodium nitroprusside 3.4mmol/L.
Reagent:- 2 Enzyme Reagent Urease> 500 U/mL., Stabilizers.
Reagent:- 3 Sodium hypochlorite 10 mmol/L, NaOH 150 mmol/L.
Standard: Urea - 50 mg/dL

Waste Management

Please refer to local regulatory requirements.

Reagent Preparation

All reagents are ready to use.

Specimen

Serum, heparin (not ammonium heparin) or urine.
Stability in serum or plasma: 7 days at 2° - 8° C
3 month at -20°C
Stability in urine: 7 days at 2° - 8°C
1 month at -20°C
For 24-hours urine storage, it should be collected in a thoroughly cleaned container which should be refrigerated during collection, measure diuresis, and take as aliquot and perform a 1:100 dilution with distilled water and calculate the amount of urea eliminated during 24 hours and multiply the results by 100.
Discard contaminated specimens

Assay Procedure

Wavelength 578 nm
Optical path 10 mm
Temperature 37 °C

PIPETTE INTO TEST TUBES

	BLANK	STD	SAMPLE
REAGENT I	1000 µl	1000 µl	1000 µl
REAGENT II	100 µl	100 µl	100 µl
STANDARD	-	10 µl	-
SAMPLE	-	-	10 µl
Mix well and incubate for 5 mins at 37°C or 10 mins at R.T.			
REAGENT III	1000 µl	1000 µl	1000 µl
Mix well and incubate for 5 mins at 37°C or 10 mins at R.T.			

Measure the absorbance (AS) of standard, (AT) of test against reagent blank at 578 nm.

Calculation

$$\text{Urea [mg/dL]} = \frac{\Delta A \text{ (Sample)}}{\Delta A \text{ (Standard)}} \times 50 \text{mg/dL}$$

Quality Controls

The reagent is linear to 200 mg/dl (33.32 mmol/L)Urea.
Samples with values above 200 mg/dl should be diluted 1:1 with 0.9% saline, reassayed and the results multiplied by 2.

Warnings and Precautions

1. **In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.**
2. **Always use safety pipettes to pull the reagents into a pipette.**
3. **Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.**
4. **The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.**
5. **For professional use only!**

Performance Characteristics

Measuring range
The test has been developed to determine urea within a measuring range from 2 - 200 mg/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Linearity/Limit of Maximum Detection

The maximum limit of detection is 200 mg/dL.

Sensitivity/Limit of Detection

The lower limit of detection is 5 mg/dL.

Specificity/Interferences

No interference was observed by, Ascorbic Acid upto 30mg/dL, Bilirubin up to 40 mg/dL, and triglycerides up to 2000 mg/dL.

Precision

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	25.51	0.52	2.03
Sample 2	42.03	0.50	1.20
Sample 3	155.96	1.22	0.78

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	24.06	0.53	2.19
Sample 2	45.41	0.59	1.31
Sample 3	146.04	0.74	0.51

Method Comparison

A comparison of Precision Biomed Urea (y) with a commercially available test (x) using 15 samples gave following results:

$y = 1.022x - 0.537$; $r^2 = 0.969$

Reference Range

Serum/Plasma	15-50 mg/dL (2.49-8.33 mmol/L)

Note: It is recommended that each laboratory should establish its own reference range based on the patient population.

Quick Reference

Parameter	Urea berthelot LQ
Mode	End Point
Wavelength	578 nm
Path length	10 mm
Standard conc.	50 mg/dL
Reagent volume	1.1 ml+1.0 ml
Sample volume	10 µL
Incubation time	5 +5 min.
Temperature	37°C
Blanking	Reagent blank
Normal range	15-50 mg/dL
Linearity	200 mg/dL
Sensitivity	5 mg/dL

Pack Size :

Cat No.	Configuration	Pack
URB00100	Reagent R1 - 1 x 50mL Reagent R2 - 1 x 5mL Reagent R3 - 1 x 50mL Standard – 1 x 2mL	2x50mL
URB01000	Reagent R1 -1 x 500mL Reagent R2 - 1 x 50mL Reagent R3 - 1 x 500mL Standard – 1 x 4mL	2x500mL

Literature

1. Fawcett, J.K. and J.E. Scott (1960). J. Clin Path. 13:156
2. Praful B. Godkar, Text Book of Medical Laboratory Technology, Bhalani Publishing House: pp. 221, 1994.
3. Thomas L. Clinical Laboratory Diagnostic. 1ed. Frankfurt: THbooks verlagsgesellschaft; 1998.p.374-7.
4. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p.1838.

Version : URB/00

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE

Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

Manufactured in India by :

Precision Biomed Pvt. Ltd.

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