Urea UV(Urease-GLDH Method)

Order Information

Cat.No.	KitConfiguration
OMR1158	Reagent 1: 2X40mL Reagent 2: 2X10mL

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.

Method

Urease –GLDH based enzymatic UV test.

Principle

Urease enzyme hydrolyses the urea into ammonia and carbon dioxide,thisammoniathenfurtherreactswithα-ketoglutaricacid. This reaction is catalyzed by Glutamate dehydrogenase (GLDH) NADH and a coloured complex is formed that can be measured by spectrophotometry.

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

$$\text{Ammonia} + \alpha\text{-ketoglutaricacid} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

Reagents Storage Instructions and Stability

Reagent and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!
Reagent 1: Enzyme Solution
Reagent 2: Substrate
Solution Standard: Urea (40 mg/dL)

Reagent Composition

Reagent - Tris 12.20 g/L, α-keto glutaric acid 3.12 g/L, Urease > 10 KU/L, GLDH (Glutamate dehydrogenase) > 1KU/L, Succinic acid 12 g/L, Albumin 1 g/L, NADH 1.10 g/L, Potassium carbonate 2.0 g/L Standard: Urea (Conc. 40 mg/dL)

Specimen

Serum, heparin (not ammonium heparin) or urine.
Stability in serumorplasma: 7 days at 2° - 8° C 3 month at-20°C
Stabilityinurine: 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

Warnings and Precautions

1. In case of contact with eyes, rinse immediately with plenty ofwater and seek medical advice.
2. Always use safety pipettes to pull the reagents into apipette.
3. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do notswallow.
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 useonly!

Waste Management

Please refer to local regulatory requirements.

Reagent Preparation

Mix, 4 parts of reagent 1 with 1 part of reagent 2 = Working reagent.
Leave the working reagent for at least 30 min. at 15° - 25°C before use.
Working Reagent Stability: 4 weeks at 2°- 8°C. Protect working reagent from light.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340nm
Opticalpath	10mm
Temperature	37°C

Substrate Start

	Blank	Sample or Standard
Sample or Standard	--	10 µL
Reagent 1	1000 µL	1000 µL
Mix Incubate 0-5 min. then add		
Reagent 2	250 µL	250 µL
Mix, incubate for approx. 30 sec. at 37°C, then read the absorbance (A1). After exactly further 60 sec. read absorbance (A2). ΔA= (A1-A2) sample/standard		

Sample Start	Blank	Sample or Standard
Sample or Standard	--	10 µL
Working Reagent	1000 µL	1000 µL
Mix, incubate for approx. 30 sec. at 37°C, then read the absorbance (A1). After exactly further 60 sec. read absorbance (A2). ΔA= (A1-A2) sample/standard		

Calculation

$$\text{Urea[mg/dL]} = \frac{\Delta A \text{ (Sample)}}{\Delta A \text{ (Standard)}} \times 40 \text{ mg/dL}$$

Conversion factor

Urea (mg/dL) x 0.1665 = Urea (mmol/L) Urea (mg/dl) x 0.467 = BUN (mg/dL) BUN (mg/dL) x 2.14 = Urea (mg/dL)

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in control recovery.

Performance Characteristics

Measuring range

The test has been developed to determine urea within a measuring range from 5 - 400 mg/dL. When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

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.

Sensitivity/Limit of Detection

The lower limit of detection is 5 mg/dL.

Linearity

The maximum limit of detection is 400 mg/dL.

Precision

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	40.81	1.14	2.78
Sample 2	127.39	1.18	0.93

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	39.21	0.85	2.16
Sample 2	125.86	3.00	2.39

Method Comparison

A comparison of Nucleus Diagnosys 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

In Serum/Plasma		
	mg/dL	mmol/L
Adults		
Global	17-43	2.8-7.2
Men <50 Years	19-44	3.2-7.3
Men >50 Years	18-55	3.0-9.2
Women <50 Years	15-40	2.6-6.7
Women >50 Years	21-43	3.5-7.2
Children		
1-3 Years	11-36	1.8-6.0
4-13 Years	15-36	2.5-6.0
14-19 Years	18-45	2.9-7.5
In Urine	26-43 g/24h	0.43-0.72 mol/24h

Each laboratory should check if the references range are transferable to its own patient population and determine own reference ranges if necessary.

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: THbooksverlagsgesellschaft;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.**

MANUFACTURED BY: NUCLEUS DIAGNOSYS LLP, INDIA

Marketed By: ORCHARD MEDICAL,

DIAMOND ARCADE, 68 JESSORE ROAD,

1st FLOOR, UNIT No. 110 & 112, KOLKATA – 700 055

CUSTOMER CARE No: 84200 69980

CUSTOMER CARE E-MAIL: sales.orchardmedical@gmail.com