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UREA

Quantitative Determination of UREA
Enzymatic UV GLDH Method

For professional in vitro diagnostic use only.

INTENDED USE

Quantitative determination of urea in Serum, Plasma or Urine. UV GLDH method.

GENERALITIES

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, prerenal azotemia, is caused by impaired perfusion of the Kidneys due to decreased cardiac output or for any of the former causes. The second, postrenal azotemia, is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinary tract.

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, whereas 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.

TEST PRINCIPLE

The urease hydrolyzes urea in sample to release ammonium ions, which react with 2-oxoglutarate and NADH in presence of glutamate dehydrogenase to form glutamate and NAD+. The decrease of absorbance is measured at 340 nm.

REAGENT COMPOSITION

R1: Enzyme Solution	
TRIS Buffer, pH 7.80	120 mmol/L
2-Oxoglutarate	7 mmol/L
ADP	0.6 mmol/L
Urease	> 6 KU/L
GLDH (Glutamate dehydrogenase)	> 1 KU/L
R2: Substrate Solution	
NADH	0.25 mmol/L
Standard	
Urea	value on label

STORAGE, PREPARATION AND SHELFLIFE

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents. Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Blank Absorbance of Working Reagent at 340 nm < 1.000 . After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge to avoid contamination, degradation from direct light and evaporation.

Working Solution preparation: Mix 4 parts of Reagent 1 with 1 part of Reagent 2. Leave the working reagent for at least 30 min. Stability: 4 weeks at 2 to 8°C, 5 days at 15 to 25°C.

SAMPLE COLLECTION AND PREPARATION

Serum or plasma, free of hemolysis and fresh urine. Other anticoagulants (ammonium heparin or double oxalate of potassium and ammonium) must not be used.

Stability: urea in serum, plasma or urine is stable 7 days at 2-8°C. Freeze for longer storage 3 months at -20°C.

Collect a 24-hour urine specimen into a plastic bottle free of preservatives, dilute urine 1:50 with distilled water. Keep the sample refrigerated to minimize urea hydrolysis by microorganisms or other agents.

Stability in urine: 2 days at 20 - 25°C; 7 days at 4 - 8°C ; 1 month at -20 ° C Discard contaminated specimens.

TEST PROCEDURE

Wavelength	: 340 nm
Light path	: 1 cm
Temperature	: 37 °C
Measurement:	: against Reagent Blank
Assay type	: Fixed Time

Assay:

	Blank	Assay
Working Solution	**1000 µL**	**1000 µL**
Sample/ Standard/ Cal	**/**	**10 µL**

- Mix and incubate for 30 seconds at 37 °C and Read Absorbance (Abs1).
- After exactly further 60 seconds read Absorbance (Abs2).
- Calculate ΔAbs (Abs2 – Abs1).

CALCULATION

Serum/ Plasma:

$$\text{Urea Concentration} = \frac{\Delta\text{Abs Sample}}{\Delta\text{Abs Std/Cal}} \times \text{Std/ Cal.}$$

Urine:

Calculate as for the serum and multiply the result by the initial sample dilution.

Notes:

- If results are to be expressed as SI units apply:

  mg/dL x 0.1665 = mmol/L

- To convert urea mass units to those of urea nitrogen apply:

  mg/dL x 0.467 = mg/dL BUN

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analyzed routinely with each group of unknown samples.

EXPECTED VALUES

Serum/plasma:

- Children (1 – 19 years): 11 - 45 mg/dL.
- Adults: 15 – 55 mg/dL.

Urine:

- Adults: 26-43 g/24h

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:

Low Level: Samples= 20; Average = 29.8; S.D. = 0.61; CV = 2.05%.
High Level: Samples = 20; Average = 117; S.D. = 1.48; CV = 1.27%.

ACCURACY:

A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y=0.971 x + 1.49 r =0.992

SENSITIVITY: 2.00 mg/dL.

LINEARITY: 300 mg/dL.

PRECAUTIONS

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing, gloves and eye/face protection.

INTERFERENCES

No interference was observed by Bilirubin up to 20 mg/dL, Hemoglobin up to 500 mg/dL and lipemia up to 1000 mg/dL Triglycerides. Ammonium ions interfere, do not use ammonium heparin as anticoagulant to collect plasma.

LITERATURE

- Falke, H.N. Schubert, G.E.Klin.Wschr.42 (1965).
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- Talke H, Schubert GE. Enzymatic determination of urea in blood and serum with the optical test according to Warburg. Klin Wschr 1965;43:174-5.
- Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
- Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

USED SYMBOLS

IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Date of Manufacture
REF	Catalogue Number
LOT	Batch Code
	Use by YYYY-MM (MM = end of month)
	Operator's Manual; Operating Instructions
	Keep away from Sunlight
	Keep away from Rain
	Temperature Limit
	Caution
	Do not use if Package is Damaged
	Do Not Re-Use
Σ	Contains Sufficient for <N> Tests