Trilogy Biotechnology LLC

225 Madinah St., 305 Commercial Complex, Amman, Jordan
Post code 11954,
Tel: +962 79 8599872,
Email: info@trilogybiotech.com
Website: www.trilogybiotech.com

UREA

Quantitative Determination of UREA
Enzymatic Colorimetric Berthelot Method

For professional in vitro diagnostic use only.

INTENDED USE

Quantitative determination of urea in Serum, Plasma or Urine. Colorimetric method according to Berthelot.

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

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside as coupling agent to yield a green chromophore. The intensity of the color formed is proportional to the concentration of urea in the sample.

REAGENT COMPOSITION

Reagent 1 (Enzyme)	
Phosphate buffer	20 mmol/L
sodium salicylate	60 mmol/L,
sodium nitroprusside	3.4 mmol/L
Urease	>20 KU/L
Reagent 2 (Alkaline)	
Sodium hypochlorite	10 mmol/L
NaOH	150 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. 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.

SAMPLE COLLECTION AND PREPARATION

Serum or heparinized plasma free of hemolysis and 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 2-8ºC. Freeze for longer storage.

Collect a 24-hour urine specimen into a plastic bottle free of preservatives. Keep the sample refrigerated to minimize urea hydrolysis by microorganisms or other agents.

TEST PROCEDURE

Wavelength	: 578 nm
Light path	: 1 cm
Temperature	: 37 °C
Measurement:	: against Reagent Blank
Assay type	: Endpoint

Assay:

	Blank	Assay
Reagent 1	**1000 µL**	**1000 µL**
Sample/ Standard/ Cal	**/**	**10 µL**

-Mix and incubate for 3 min at 37 °C or for 10 min at room temperature (16-25 °C).

-Add

Reagent 2	**1000 µL**	**1000 µL**

-Mix and incubate for 5 min at 37 °C or for 10 min at room temperature (16-25 °C).

- Read the absorbance (A) of the samples and the standard against the reagent blank.

CALCULATION

Serum/ Plasma:

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

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:

- Newborns (<10: days): 6.4 - 53.5 mg/dL
- Adults: 15 - 40 mg/

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 = 62.3; S.D. = 2.18; CV = 3.33%.
High Level: Samples = 20; Average = 141.06; S.D. = 5.76; CV = 4.28%.

ACCURACY:

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

y=0.923 x + 0.4987 r =0.99

SENSITIVITY: 0.00 mg/dL.

LINEARITY: 350 mg/dL.

PRECAUTIONS

R2: contains Sodium Hydroxide.

in case of contact of reagents with the operator, you must apply the following first aid:

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: Hemoglobin up to 200 mg/dL, Bilirubin up to 4,0 mg/dL and lipemia up to 200 mg/dL Triglycerides.

LITERATURE

- Chaney, A.L., and Marbach, E.P. Clin. Chem. 8 : 132 (1962).
- Searcy, R.L., Reardon, J.E., and Foreman, J.A. Am. J. Clin. Technol. 33 : 15-20 (1967).
- Young DS. Effects of drugs on clinical laboratory tests, 5thed. AACC Press, 2000.
- Tietz. N.W. Clinical Guide to Laboratory Tests, 3rdEdition. W.B. Saunders Co. Philadelphia, PA. (1995).
- Friedman and Young. Effects of disease on clinical laboratory tests. 5th ed. AACC (Press 2000).

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