Urea (Berthelot)

Urease Berthelot method for determination of urea in Serum or Plasma or Urine

2x60 mL,6X100 mL

Clinical Significance

Urea is a nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemias, caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent. In renal diseases urea concentrations are elevated when the glomerular filtration rate is markedly reduce and when the protein intake is higher than 200g/day.

Principle

Urea is converted quantitatively by urease into ammonia and carbon di oxide. In this modified method ammonium ions reacts with hypochlorite and salicylate to give a green colored complex (Berthelot reaction). The color yield is enhanced by Sodium nitroprusside. The color intensity is directly related to the urea concentration and is measured photometrically at 578 nm.

REAGENTS PROVIDED

Urease	:30,000 U/L
Sodium Hypochlorite	:10 mmol/L
NaoH	:400 mmol/L
Soidum Salicylate	:40 mmol/L
Activator & Stabilizers	
Standard	**:**40 mg/dL

Precautions : For in vitro use only

Reagents are ready to use for the given procedure.

Working reagent preparation

A working enzyme reagent may be made by pouring 1 bottle R2 (Enzyme reagent) into 1 bottle of R1 (Buffer reagent).

Working reagent is stable for 16 weeks when proper storage conditions are strictly maintained.

Stability and Storage

The unopened reagents are stable till the date of expiry mentioned on the labels when stored at 2 to 8 °C.

Instability or Deterioration of Reagents

Visible turbidity in reagent or standard indicates reagent deterioration.

Specimens

Sample: Serum, plasma or urine.

a) Collection: obtain non hemolysed serum in the usual ways. As serum age's ammonia is produced. The equilibration time may be increased to compensate for excess ammonia. Urea in serum is stable for 1 day at RT and 72 hours at 2°–8 °C. Plasma should be collected in ammonia salt free anticoagulant.

b) 24 hour urine should be collected in a thoroughly clean container, which should be refrigerated (2°-10C) during collection. Measure diuresis, take an aliquot and perform a 1:100 dilution calculate the amount of urea eliminated during 24 hours.

c) Additives: non-required

For detailed interferences refer to Young et al[3]

Assay Procedure

Wavelength : 570 nm

Temperature : 37 °C

Light Path : 1 cm

1. Prepare working reagent as per instructions. Bring all reagents to R.T.
2. Pipette into tubes neat Serum/Plasma.

	B	S	U
Working Reagent (R1+R2)	1000 µL	1000 µL	1000 µL
Standard	--	10 µL	--
Unknown sample	--	--	10 µL

3. Mix, incubate for 3 min. at 37 °C (5 Min at RT), and then add

Reagent R3	1000 µL	1000 µL	1000 µL

4. Mix, incubate for 5 min. at 37 °C (10 Minutes at RT).
5. At the end of incubation measure the absorbance of the (AS), Unknown (AU), against blank at 570 nm.
6. Determine the result; the final color is stable for at least 10 hrs at RT.

Application sheets for automated systems are available on request

Calculation

Serum/Plasma

$$\text{Urea} = \frac{AU}{AS} \times 40 \text{ (Standard Conc.)}$$

- To convert result to mmol/L multiply Urea mg/dL by 0.167
- To convert result to Blood urea nitrogen multiply Urea mg/dl by 0.467

Reference Values

Serum : 14 – 40 mg/dL

Urine : Up to 20g/L

According to the IFCC (International federation of clinical chemistry) recommendations, each laboratory should set its own intervals or reference values among a group or population of patients selected by relevant criteria (age, sex, life habits etc.) and using the method under study.

Quality Control

The use of Commercial controls sera is recommended with each assays batch to monitor procedural parameters. Use 2 controls containing normal and abnormal levels when possible.

Procedure Limitations

Method is linear up to 300 mg/dL. Samples with Urea concentration above 300 mg/dL should be suitably diluted, re-assayed as above and the final results should be multiplied with the appropriate dilution factor to get the final value

Note;

- Enzyme reagent (R2) may appear slightly hazy,but after mixing it with Buffer reagent (R1) its haziness disappears and this does not affect the perfomance of kit.
- Any contamination by ammonia or ammonium salts lead to erroneous results; hence plasma should not be collected within Flouride or Heparin ammonium salts.
- The working enzyme reagent is not stable at elevated temperatures and should be stored back at 2 to 8º C immediately after use.
- The Chromogen reagent contains Chlorine.The bottles should be opened only when required and closed tightly after use to prevent the loss of active chlorine.

SYSTEM PARAMETERS		
Mode	:	End point
Reaction	:	Ascending
Wavelength	:	570 nm (570 – 620)
Blank with	:	Reagent
Sample Volume	:	10 µL
Reagent Volume	:	2000 µL
Standard	:	40 mg/dL
Incubation Time	:	5 min + 3 min
Linearity	:	300 mg/dL
Unit	:	mg/dL

Literature

1. Trinder P. Ann. Clin. Biochem 6:24 (1969)
2. Emmerson E.J. et.al. J.org. Chem.153(1938) and 8:417 (1943)
3. Young DS et. al. Clin Chem 21:1D (1975)

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