UREA

Berthelot/Colorimetric Method

PRODUCT CODE
CS017

INTENDED USE
For the quantitative determination of Urea in serum, plasma & urine.

CLINICAL SIGNIFICANCE
Urea is the end product of the protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy

PRINCIPLE
Urease catalyses the conversion of urea to ammonia. In a modified Berthelot reaction, the ammonium ions react with a mixture of salicylate, hypochlorite and nitroprusside to yield a blue-green dye (Indophenol.) The intensity of this dye is proportional to the concentration of urea in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \text{salicylate} + \text{hypochlorite} \xrightarrow{\text{Nitroprusside}} \text{2, 2-Dicarboxyindophenol}$$

REAGENT COMPOSITION

UREA REAGENT 1

Phosphate buffer	120 mmol/L
Sodium Salicylate	60 mmol/L
Sodium nitroprusside	5 mmol/L
EDTA	1 mmol/L
Urease	5 KU/L

UREA REAGENT 2

Phosphate buffer	120 mmol/L
Sodium Hydroxide	400 mmol/L
Sodium Hypochlorite	10 mmol/L

UREA STANDARD

Urea standard concentration	80 mg/dL or 13.3mmol/L

REAGENT PREPARATION
Reagents and standard are ready for use.

REAGENT STORAGE AND STABILITY
The reagents and standard are stable up to the expiry date when stored at 2 - 8° C.

SPECIMEN
Serum, Plasma (provided the anticoagulant used does not contain ammonium or fluoride) and urine 24hrs dilute (1 urine + 100 distilled water) Urea in serum is stable at 2-8° C for 3 days. Do not use lipaemic samples.

PRECAUTION
To avoid contamination, use clean laboratory wares.
Serum specimens should be considered infectious and handled appropriately.

ASSAY

Wavelength	578 nm
Cuvette	1 cm light path
Temperature	20-25°C, or 37°C
Measurement	Against reagent blank

PROCEDURE

Pipette into cuvettes	Blank	Standard	Sample
Reagent-1	1000 µL	1000 µL	1000 µL
Sample	--	--	10 µL
Standard	--	10 µL	--
Mix and incubate for 5 minutes at 20-25°C or 3 minutes at 37°C			
Reagent-2	1000 µL	1000 µL	1000 µL
Mix and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C Measure the absorbance of the sample (As) and the standard (Astd) against the reagent blank			

CALCULATION

$$\text{Urea Conc. (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 80 \text{ (Std.conc.)}$$

Urea (g/24 urine) = mg/dL X volume of 24-hour urine
To convert mg/dL to mmol/L divide by 6.01

LINEARITY
Serum values up to 400 mg / dL or 66.6 mmol/L. Urine values up to 40 g/1 or 6.66 mol/l
For higher values dilute sample 1+1 with distilled water, repeat assay and multiply the results by 2.

APPLICATION ON AUTO BIOCHEMISTRY ANALYZERS

BR-160 AUTO BIOCHEMISTRY ANALYZER

TEST NAME	UREA			SAMPLE VOLUME	2	
FULL NAME	UREA					
				REAGENT VOLUME	R1 150	R2 150
WAVE LENGTH	PRI. 578	SEC. NONE				
ASSAY /POINT	1 POINT END	START 1	END 25			
DECIMAL PLACE	2					
UNIT	mg/dl					
LINEARITY RANGE	LOW 0	HIGH 400				

BR-120 AUTO BIOCHEMISTRY ANALYZER

TEST NAME	UREA			SAMPLE VOLUME	2	
FULL NAME	UREA					
				REAGENT VOLUME	R1 150	R2 150
WAVE LENGTH	PRI. 578	SEC. NONE				
ASSAY /POINT	1 POINT END	START 6	END 39			
DECIMAL PLACE	2					
UNIT	mg/dl					
LINEARITY RANGE	LOW 0	HIGH 400				

PERFORMANCE CHARACHTERISTICS

Measuring range:
From the detection limit to the linearity limit of 0,743 mg/dl to linearity limit of 400 mg/dl.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Sensitivity: 1 mmg/dl = 0, 00180 A.
Precision:

Description	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dl)	28.50	70.60	29.35	70.45
SD	1.88	3.65	1.69	2.63
CV (%)	6.59	5.17	5.75	3.73

Accuracy:

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Results obtained using Bio Research reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 30 samples were the following:
Correlation coefficient $(r)^2$: 0.779
Regression equation: y= 0.7527x + 7.38
The results of the performance characteristics depend on the analyzer used

NORMAL RANGE

Serum	10 - 50 mg/dL	1.66 - 8.30 mmol/L
Urine 24hrs	10 – 35 g/L	1.66 - 5.83 mol/L

QUALITY CONTROL
All control sera with Urea values estimated by this method can be used.

NOTES
1- The test is not influenced by haemoglobin values up to 200 mg/dL or by bilirubin values up to 10mg/dL.
2- The standard contains sodium azide (0.1%) as preservative. Do not swallow and avoid contact with skin and mucous membranes.
3- Sodium hydroxide and hypochlorite in reagent 2 are irritants. In case of contact with eyes or mucous membranes wash immediately with water.
4- 1mg of urea corresponds to 0.467 mg of urea nitrogen.

SYMBOL ON LABELS

Symbol	Signify	Symbol	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative		

BIBILOGRAPHY
1- Berthelot A et at Clin. Chem 25 (2), 336, 1979
2- Tobacco, A et at , Clin, Chem 25 (2), 336 , 1979
3- Chaney A. L and Marbach E.P., Clin. Chem. 8.130 . 1962

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