Urea

Urease GLDH. Kinetic. Liquid

Quantitative determination of urea

IVD

PACKAGING

Ref.: 101-0439	Cont.: 4 x 160 / 4 x 40 mL
Ref.: 101-0575	Cont.: 4 x 50 / 1 x 50 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

Urea is the final result of the metabolism of proteins; it is formed in the liver from its destruction. Elevated urea can appear in blood (uremia) in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction[1,6,7].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Urea in the sample is hydrolized enzymatically into ammonia (NH_4^+) and carbon dioxide (CO_2).

Ammonia ions formed react with α-ketoglutarate in a reaction catalysed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD+:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2$$

$$NH4^+ + \alpha\text{- Ketoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

The intensity of the color formed is proportional to the urea concentration in the sample[1,2,3].

REAGENTS

R 1 Buffer	Tris pH 7.8	80 mmol/L
	A-Ketoglutarate	6 mmol/L
	Urease	75000 U/L
R 2 Enzymes	GLDH	60000 U/L
	NADH	0.32 mmol/L
UREA CAL	Urea aqueous primary standard 50 mg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Working reagent (WR): Mix **4 vol. R1 Buffer + 1 vol. R2 Substrate.** The (WR) is stable for 1 month at 2-8º C or 1 week at room temperature (15-25º C).

UREA CAL: Ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm $\leq$ 1.00

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment (Note 1).

SAMPLES

- Serum or heparinized plasma[1]: Do not use ammonium salts or fluoride as anticoagulants.
- Urine[1]: Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor). Preserve urine samples at pH < 4.

Urea is stable at 2-8º C for 5 days;

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

UREA CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Glassware and distilled water must be free of ammonia and ammonium salts[1].

Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . 1 cm light path
   Temperature. . . . . 37º C / 15-25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard (Note 2-3) (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and read the absorbance after 30 s (A_1) and 90 s (A_2).
5. Calculate $\Delta A = A_1 - A_2$

CALCULATIONS

$$\frac{(A_1 - A_2)\,Sample - (A_1 - A_2)\,Blank}{(A_1 - A_2)\,Standard - (A_1 - A_2)\,Blank} \times 50\ (\text{Calibrator conc.}) = \text{mg/dL urea in the sample}$$

10 mg/L urea BUN divided by 0.466 = 21 mg/L urea = 0.36 mmol/L urea[1].

Conversion factor: mg/dL x 0.1665 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum : 15- 45 mg/dL (2.49 - 7.49 mmol/L)

Urine : 20 - 35 gr/24 h.

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit 0.743 mg/dL to linearity limit 433 mg/dL.

If the concentration is greater than linearity limit dilute 1:2 the sample with NaCl 9 g/L and multiply the result by 2.

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Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	37.5	120	40.0	126
SD	1.05	0.92	1.06	2.07
CV (%)	2.79	0.77	2.65	1.65

Sensitivity: 1 mg/dL = 0.00180 A.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.98209.
Regression equation: y= 1.0343x – 1.2105.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
It is recommended to use heparin as anticoagulant. Do not use ammonium salts or fluoride[1].
A list of drugs and other interfering substances with urea determination has been reported by Young et. al[4,5].

BIBLIOGRAPHY
1. Kaplan A. Urea. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1257-1260 and 437 and 418.
2. Tabacco A et al. Cin Chem 1979; 25: 336-337.
3. Fawcett J K et al. J Clin Path 1960; 13: 156-169.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
6. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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