CHRONOLAB

CE
IVD

Urea-UV

Urease -GLDH. Kinetic UV

Quantitative determination of urea

PACKAGING

Ref: 101-0294	Cont.: 5 x 20 mL
Ref: 101-0274	Cont.: 4 x 50 mL
Ref: 101-0206	Cont.: 12 x 50 mL
Ref: 101-0449	Cont.: 8 x 100 mL

Store at 2-8ºC

CLINICAL SIGNIFICANCE

Urea is the final result of the metabolism of proteins; it is formed in the liver from its destruction.

It can appear elevated urea in blood (uremia) in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction[1,4,5].

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_3) 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^+:

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

$$2\ NH_3 + \alpha\text{ - Ketoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

The decrease in concentration of NADH, is proportional to urea concentration in the sample[1].

REAGENTS

R 1 Buffer	TRIS pH 7.8	80 mmol/L
	α -Ketoglutarate	6 mmol/L
R 2 Enzymes	Urease	3750 U/L
	Glutamate dehydrogenase (GLDH)	6000 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): Dissolve (→) the content of vial R 2 Enzymes into the corresponding volume of R 1 Buffer.

Cap and mix gently to dissolve contents.

Stable: 6 weeks at 2-8º C or 7 days at 15-25º C.

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 < 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 with 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 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 contaminated 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

$$\frac{(\Delta A)\ Sample - (\Delta A)\ Blank}{(\Delta A)\ S\tan dard - (\Delta A)\ Blank} \times 50\ (\text{Standard conc.}) = \text{mg/dL urea in the sample}$$

mg/dL urea x 0.466 = mg/dL urea BUN (Blood Urea Nitrogen)

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.

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PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 1.241 mg/dL to *linearity limit* of 530 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.

Precision:

	Intra-assay (n=20)	
Mean (mg/dL)	40.7	130
SD	0.88	1.02
CV (%)	2.16	1.78

Inter-assay (n=20)	
40.5	128
1.19	2.07
2.94	1.61

Sensitivity: 1 mg/dL = 0.00080 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): 0.998.

Regression equation: y=1,5759x + 1.1577.

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 [2,3].

BIBLIOGRAPHY

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