CE
IVD

Urea

Berthelot. Enzymatic colorimetric

Quantitative determination of urea

PACKAGING

Ref: 101-0425	Cont.: 2 x 100 mL
Ref: 101-0248	Cont.: 4 x 250 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 reacts with salicylate and hypochlorite (NaClO), in presence of the catalyst nitroprusside, to form a green indophenol:

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

$$NH_4^+ + \text{Salycilate} + \text{NaClO} \xrightarrow{\text{Nitroprusside}} \text{Indophenol}$$

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

REAGENTS

R 1 Buffer	Phosphate pH 6.7	50 mmol/L
	EDTA	2 mmol/L
	Sodium salicylate	400 mmol/L
	Sodium nitroprusside	10 mmol/L
R 2 NaClO	Sodium hypochlorite (NaClO)	140 mmol/L
	Sodium hydroxide	150 mmol/L
R 3 Enzymes	Urease	30000 U/L
UREA CAL	Urea aqueous primary standard 50 mg/dL	

PRECAUTIONS

R2: H314-Causes severe skin burns and eye damage.

Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

- Working reagent (WR): Dissolve one tablet R 3 Enzymes in one bottle of R 1 Buffer. Cap and mix gently to dissolve contents.
  Stability: 4 weeks in the refrigerator (2-8º C) or 7 days at room temperature (15-25º C).
- R 2 NaClO is 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 580 nm $\geq$ 0.32.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 580 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: . . . . . 580 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 incubate 5 min at 37º C or 10 min at room temperature (15-25º C).
5. Pipette:

	Blank	Standard	Sample
R 2 (mL)	1.0	1.0	1.0

6. Mix and incubate 5 min at 37º C or 10 min at room temperature (15-25º C).
7. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 30 minutes at 15-25º C.

CALCULATIONS

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

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: Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253).

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* of 0,001mg/dL to *linearity limit* of 225 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)		Inter-assay (n=20)	
Mean (mg/dL)	39	126	40,0	127
SD	0,55	2,12	0,93	2,48
CV (%)	1,43	1,68	2,33	1,96

Sensitivity: 1 mg/dL = 0,00608 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 $(r)^2$: 0,99143.

Regression equation: y= 1,0476x – 0,2846

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

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