SPINREACT

CE UREA -37

Urea

o-Phthalaldehyde 37°C. Colorimetric

Quantitative determination of urea
IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD
Urea in the sample reacts with o-fhthalaldehyde in acid medium forming a coloured complex that can be measured by spectrophotometry:

$$\text{Urea} + \text{o-fhthalaldehyde} \xrightarrow{H^+} \text{Isoindoline}$$

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

CLINICAL SIGNIFICANCE
Urea is the final result of the metabolism of proteins. It is formed in the liver from their destruction.
It can appear the urea elevated 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.

REAGENTS

R 1	o-Phthalaldehyde	4.8 mmol/L
R 2	Borate solution	87 mmol/L
	Sulphuric acid	3 mol/L
UREA CAL	Urea aqueous primary standard 50 mg/dL	

PRECAUTION
R2: Corrosive (C). R35: Causes severe burns. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S30: Never add water to this product. S45: In case of accident or if you feel unwell, seek medical advice immediately.

PREPARATION
All the reagents are 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 2-8°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 510 nm ≥ 0.20.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 510 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 the results by 50 (dilution factor). Preserve urine samples at pH < 4.

Urea is stable at 2-8°C for 5 days.

PROCEDURE AND CALCULATIONS
1. Assay conditions:
   Wavelength: ..... 510 (500-550) nm
   Cuvette: ..... 1 cm light path
   Temperature ..... 37°C.
2. Adjust the instrument to zero with distilled water.

A) Kinetic method
1. Pipette into a cuvette:

	Blank	Standard	Sample
R 1 (mL)	1.0	1.0	1.0
Standard[Note 2-3] (µL)	--	50	--
Sample (µL)	--	--	50

2. Mix, wait 1 minute and add:

R 2 (mL)	1.0	1.0	1.0

3. Mix. Incubate at 37°C and read the absorbance after 1 minute (A_1) and after 2 minutes (A_2).
4. Calculate the increase of the absorbance $\Delta A = A_2 - A_1$.

Calculations

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

B) End point
1. Pipette into a cuvette:

	Blank	Standard	Sample
R 1 (mL)	1.0	1.0	1.0
Standard[Note 2-3] (µL)	--	25	--
Sample (µL)	--	--	25

2. Mix, and add:

R 2 (mL)	1.0	1.0	1.0

3. Mix and incubate 15 min at 37°C.
4. Read the absorbance (A) against the Blank.

Calculations

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

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: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
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 0.70 mg/dL to linearity limit of 200 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)	43.2	145	41.9	147
SD	1.51	1.10	0.80	2.83
CV (%)	3.49	0.76	1.92	1.91

Sensitivity: 1 mg/dL = 0.00459 A.
Accuracy: Results obtained using SPINREACT 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): 0.9884
Regression equation: $y = 0.975x + 0.6$
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[2,3].

NOTES
1. UREA CAL: Proceed carefully with this product because due its nature it can get contamined easily.
2. Glassware and distilled water must be free of ammonia and ammonium salts[1].
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.
5. SPINREACT has instruction sheets for several automatic analyzers; instructions for many of them are available on request.

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 416.
2. Young DS. Effects of drugs on Clinical Lab. Tests. 4th ed AACC Press. 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests. 4th ed AACC 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry. 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests. 3rd ed AACC 1995.

PACKAGING

Ref. 1001323	Cont.	2 x 50 mL
Ref. 1001325		2 x 250 mL
Ref. 1001326		2 x 100 mL

SPINREACT,S.A.U Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99 e-mail: spinreact@spinreact.com