Reactivos GPL

Barcelona, España

CE

-UREA-37-

UREA

o-Phthalaldehyde 37ºC. Colorimetric.

Store at: +2+8ºC.

Presentation:
Cod. SU040 CONT: *R1 1 x 125 mL.+ R2 1 x 125 mL.+ Cal. 1 x 5 mL.*

Procedure

Quantitative determination of urea.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
Urea in the sample reacts with *o*-fhthalaldehyde in acid medium forming a coloured complex that can be measured by spectrophotometry:

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

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

REAGENTS COMPOSITION

R.1	*o*-Phthalaldehyde	4.8 mmol/L
R.2	Borate solution	87 mmol/L
Calibrator	Urea aqueous primary standard	50 mg/dL

REAGENT PREPARATION AND STABILITY
All the reagents are ready to use.
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contamination prevented during their use.
Do not use reagents over the expiration date.
Urea Cal.: Once open is stable up to 1 month when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 510 nm $\geq$ 0.20

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-8ºC,. Do not use reagents over the expiration date.

SPECIMEN
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 2-8ºC for 5 days.

MATERIAL REQUIRED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 510 nm.
- Matched cuvettes 1.0 cm. light path.
- General laboratory equipment (Note 1).

TEST PROCEDURE
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 37ºC 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

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

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, wait 1 min and add:

R 2 (mL)	1.0	1.0	1.0

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

CALCULATIONS

$$\text{Urea mg/dL} = \frac{(A)\text{Sample}}{(A)\text{Calibrator}} \times 50 \text{ (Calibrator conc.)}$$

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

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or Plasma	
15-45 mg/dL	2.5-7.5 mmol/L
Urine	
20-35 g/24 h	

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

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

REAGENT PERFORMANCE
– *Measuring Range*: From detection limit of 0.70 mg/dL. to linearity limit of 200 mg/dL., under the described assay conditions.
If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply 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 GPL reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES
– Interference:
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. Glassware and distilled water must be free of ammonia and ammonium salts[1].
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

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