Reactivos GPL

Barcelona, España

CE

- UREA-B -

UREA
Berthelot Enzymatic

Store at: +2+8ºC.

Presentation:
Cod. SU038 CONT: *R 2 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 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 COMPOSITION

R.1 (Buffer)	Phosphate pH 6.7 EDTA Sodium salicylate Sodium nitroprusside	50 mmol/L 2 mmol/L 60 mmol/L 3.2 mmol/L
R.2 (NaCLO)	Sodium hypochlorite (NaClO) Sodium hydroxide	140 mmol/L 150 mmol/L
R.3 (Enzymes)	Urease	30000 U/L
Calibrator	Urea aqueous primary standard	50 mg/dL.

PRECAUTIONS

R 2: Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

REAGENT PREPARATION AND STABILITY

- 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 (2-8ºC) or 7 days at room temperature (15-25ºC).

- R 2 NaClO is 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 580 nm ≥ 0.32.

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 580 nm.
- Matched cuvettes 1.0 cm. light path.
- General laboratory equipment (Note 1).

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions
- Wavelenght : ..... 580 nm. (570-590).
- Cuvette: ..... 1 cm light path.
- Temperature .....37ºC. 15-25ºC.

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Calibrator	Sample
WR (mL.)	0.5	0.5	0.5
Calibrator(note1-2) (µL.)	--	5	--
Sample (µL.)	--	--	5

4. Mix and incubate 5 min at 37ºC or 10 min at room temperature (15-25ºC).
5. Pipette:

	Blank	Calibrator	Sample
R.2 (mL.)	0.5	0.5	0.5

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

CALCULATIONS

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

mg/dL of urea x 0.466 = 21 mg/dL of urea / BUN (Blood Urea Nitrogen)[1].

Conversion Factor: mg/dL. x 0.1665 = mmol/dL.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control Normal Ref. QC003 and Control Pathological Ref. QC004. 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

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 0.3 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 (g/dL)	40	139	40	142
SD	1.27	3.50	1.86	3.75
CV	3.17	2.50	4.64	2.63

- Sensitivity:
  1 mg/dL. = 0.00505 A
- Accuracy:
  Results obtained GPL 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): 0.9941
  Regression Equation: y= 0.9972x + 0.011
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

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].

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.

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