



Store at: +2+8° C.

Presentation:

Cod. SU037-SP CONT: R1 1 x 40mL. R2 1 x 10 mL. + CAL 1 x 5 mL
 Cod. SU036 CONT: R1 1 x 100 mL. R2 1 x 25 mL. + CAL 1 x 5 mL
 Cod. SU039 CONT: R1 2 x 100 mL. R2 2 x 25 mL. + CAL 1 x 5 mL

Procedure

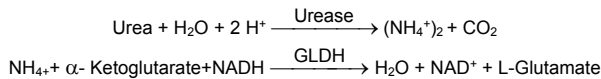
Quantitative determination of urea

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

TEST SUMMARY

Urea in the sample is hydrolyzed enzymatically into ammonia (NH₄⁺) and carbon dioxide (CO₂).

Ammonia ions formed reacts with α-ketoglutarate in a reaction catalysed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD⁺:



The decrease in concentration of NADH, is proportional to urea concentration in the sample¹.

REAGENTS COMPOSITION

R 1	TRIS pH 7.8	80 mmol/L
Buffer	α-Ketoglutarate	6 mmol/L
	Urease	75000 U/L
R 2	GLDH	60000 U/L
Enzymes	NADH	0.32 mmol/L
UREA CAL	Urea aqueous primary standard 50 mg/dL	

REAGENT PREPARATION AND STABILITY

Working reagent (WR)

Mix: 4 vol. R1 Buffer + 1 vol. R2 Substrate.

The (WR) is stably for 1 month at 2-8°C or 1 week at room temperature (15-25°C).

UREA CAL: Ready to use.

Proceed carefully with this product because due its nature it can get contaminated easily.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.00.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

SPECIMEN

- Serum or heparinized plasma¹: Do not use ammonium salts or fluoride as anticoagulants.
- Urine¹: 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.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched cuvettes 1.0 cm light path.

General laboratory equipment.

TEST PROCEDURE

1. Assay conditions:
 - Wavelength: 340 nm
 - Cuvette: 1 cm light path
 - Temperature 37°C / 15-25°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 (A₁) and 90 s (A₂). Calculate: ΔA = A₁ - A₂.

CALCULATIONS

$$\frac{(A_1 - A_2) \text{ Sample} - (A_1 - A_2) \text{ Blanco}}{(A_1 - A_2) \text{ Calibrator} - (A_1 - A_2) \text{ Blanco}} \times 50 \text{ (Calibrator conc)} = \text{mg/dL urea}$$

 in the sample

10 mg/L urea BUN divided by 0.466 = 21 mg/L urea = 0.36 mmol/L urea¹.

Conversion factor: mg/dL x 0.1665 = mmol/L.

QUALITY CONTROL

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

Serum or plasma:

15-45 mg/dL ≅ 2.5-7.5 mmol/L

Urine:

26 - 43 g/24 h ≅ 428-714 mmol/24h

(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 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.

REAGENT PERFORMANCE

- **Measuring range:** From detection limit 0.743 mg/dL to linearity limit 400 mg/dL.

If the concentration is greater than linearity limit dilute 1:2 the sample with ClNa 9 g/L and multiply the result by 2.

- **Precision:**

Mean (mg/dL)	Intra-assay (n=20)		Inter-assay (n=20)	
	SD	CV (%)	SD	CV (%)
37.5	1.05	2.79	40.0	2.65
120	0.92	0.77	126	1.65

- **Sensitivity:** 1 mg/dL = 0.00180 A.

- **Accuracy:** Results obtained using GPL reagents (y) did not show systematic differences when compared with other commercial reagent (x).

The results obtained using 50 samples was the following:

Correlation coefficient (r): 0.98209.

Regression equation y = 1.0343x - 1.2105

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 fluoride¹.

A list of drugs and other interfering substances with urea determination has been reported^{2,3}.

NOTES

1. Glassware and distilled water must be free of ammonia and ammonium salts¹.
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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