Urea MonlabTest®

Urease-GLDH. Kinetic. Liquid.

Quantitative determination of urea.

IVD Only for professional in vitro diagnostic use

Store at 2-8°C

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

$$\text{Urea} + H_2O + 2\,H^+ \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2$$

$$NH_{4+} + \alpha\text{- Ketoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

The decrease in concentration of NADH, is proportional to 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 Buffer	TRIS pH 7.8	80 mmol/L
	α-Ketoglutarate	6 mmol/L
	Urease	75000 U/L
R 2 Enzymes	GLDH	60000 U/L
	NADH	0,32 mmol/L
UREA CAL	Urea aqueous primary standard 50 mg/dL	

PREPARATION

Working reagent (WR): Mix **4 vol. R1 Buffer + 1 vol. R2 Substrate.**

The (WR) is stably for 1 month at 2-8ºC.

UREA CAL: 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 2-8ºC, 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 340 nm < 1.00.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 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 2-8ºC for 5 days.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . .1 cm light path
   Temperature . . . . . 37ºC / 15-25ºC
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 (A_1) and 90 s (A_2).
5. Calculate: $\Delta A = A_1 - A_2$.

CALCULATIONS

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

10 mg/L urea BUN divided by 0,466=21mg/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: Normal and Pathologic CONTROL (MO-165107 and MO-165108).

If control values are found outside the defined range, check the instrument, reagent and calibration for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[4,5]

Serum or plasma:

15-45 mg/dL ≅ 2,5-7,5 mmol/L

Urine:

26 – 43 g/24 h ≅ 428-714 mmol/24 h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range*:* From detection limit 0,743 mg/dL to linearity limit 433 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:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	37,5	120	40,0	126
SD	1,05	0,92	1,06	2,07
CV (%)	2,79	0,77	2,65	1,65

Sensitivity: 1 mg/dL = 0,00180 A.

Accuracy: Results obtained using MONLABTEST 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.

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[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].

Ref: MO-165101/102
Rev: JANUARY 2013

Manufacturer: Monlab SL Selva de Mar 48 08019 BCN Spain + 34 93 433 58 60 Fax +34 93 436 38 94 pedidos@monlab.com www.monlab.com

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. **MONLAB 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 418.
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.: MO-165101 R1: 1 x 40 mL R2: 1 x 10 mL UREA CAL: 1 x 2 mL	Ref: MO-165102 R1: 1 x 1000 mL R2: 1 x 250 mL UREA CAL: 1 x 5 mL

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	Manufacturer	IVD	For i*n vitro* diagnostic use only
	Don't re-use		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by

Manufacturer: Monlab SL Selva de Mar 48 08019 BCN Spain + 34 93 433 58 60 Fax +34 93 436 38 94 pedidos@monlab.com www.monlab.com