LIQUID LINE

Urea UV

cinética AA

For urea determination in serum, plasma or urine

SUMMARY

Urea constitutes the most important fraction of non-protein nitrogen present in most biological fluids. It is the main end product of protein metabolism in human beings. It is produced in the liver and is eliminated from the body by urine through the kidneys.

An increase in the concentration of serum urea is generally interpreted as a possible renal dysfunction. However, it should be considered that urea serum values are closely related to diet and protein metabolism, therefore any alteration in these variables will result in a change in the concentration of serum urea.

PRINCIPLE

The reaction system is as follows:

$$\text{urea} + H_2O \xrightarrow{\text{urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{\text{GIDH}} \text{l-glutamate} + NAD^+ + H_2O$$

PROVIDED REAGENTS

S. Standard*: 0.60 g/l urea solution (equivalent to 28.04 mg/dl BUN).

A. Reagent A: solution containing Good buffer pH 7.6, 2-oxoglutarate, urease and glutamate dehydrogenase (GIDH).

B. Reagent B: NADH solution.

Final concentrations

Good buffer	250 mmol/l
2-Oxoglutarate	7.5 mmol/l
NADH	0.28 mmol/l
Urease (Jack bean)	≥ 5,000 U/l
GIDH (microbial)	≥ 800 U/l

NON-PROVIDED REAGENTS

Wiener lab.'s **Calibrador A Plus**.

INSTRUCTIONS FOR USE

Standard: ready to use.

Reagents A and B: ready to use. They can be used separately or as a **Monoreagent** mixing 4 parts of Reagent A + 1 part of Reagent B (e.g. 4 ml Reagent A + 1 ml Reagent B).

WARNINGS

Reagents are for "in vitro" diagnostic use.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box. Once opened, they should not remain opened and outside the refrigerator for long periods of time. Avoid contamination.

Monoreagent (premixed): stable in refrigerator (2-10ºC) for 30 days from preparation date.

INSTABILITY OR DETERIORATION OF REAGENTS

Turbidity indicates deterioration of reagents.

When spectrophotometer has been set to zero with distilled water, Blank absorbance readings bellow 1.000 O.D. (at 340 nm) indicate the reagent deterioration.

SAMPLE

Serum, plasma or urine

a) Collection: obtain serum in the usual way, or plasma collected with ordinary anticoagulants. Separate from red blood cells within 24 hours from the sample collection. If urine is used, it should be preferably fresh.

b) Additives: if plasma is used as sample, collection with heparin or EDTA (Wiener lab.'s **Anticoagulante W**), is recommended.

c) Known interfering substances: no interferences are observed from: bilirubin up to 150 mg/l hemoglobin up to 350 mg/dl and triglycerides up to 7 g/l.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: serum urea is stable for up to 7 days at 20-25ºC or 2-10ºC or up to 12 months at -20ºC without preservatives. Urine urea is stable for up to 2 days at 20-25ºC, 7 days at 2-10ºC or up to 4 weeks at -20ºC without preservatives.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer
- Micropipettes and pipettes for measuring the stated volumes
- Spectrophotometric cuvettes
- Stopwatch

ASSAY CONDITIONS

(absorbance decrease)

- Wavelength: 340 nm (Hg 334 or 366)
- Reaction temperature: 37ºC
- Reaction time: 2 minutes

** Non-provided with all kit sizes*

- Sample volume: 10 ul
- Final reaction volume: 1.01 ml

Sample and Reagent volumes may be varied proportionally in order to meet the requirements of different spectrophotometers.

PROCEDURE

I- SEPARATE REAGENTS' TECHNIQUE

Set spectrophotometer to zero O.D. with distilled water. In a cuvette at the selected temperature, place:

Reagent A	1 ml
Sample or **Standard**	10 ul

Mix without inversion. Incubate for approximately 1 minute at 37ºC. Then add:

Reagent B	250 ul

Mix without inversion and simultaneously start the stopwatch. Read absorbance (U_1 or S_1) at exactly 60 seconds and continue incubation. Read absorbance again (U_2 or S_2) at exactly 120 seconds (60 seconds after first reading).

II- MONOREAGENT TECHNIQUE

Set spectrophotometer to zero O.D. with distilled water. In a cuvette at the selected temperature, place:

Monoreagent	1 ml
Sample or **Standard**	10 ul

Mix without inversion and simultaneously start the stopwatch. Read absorbance (U_1 or S_1) at exactly 60 seconds and continue incubation. Read absorbance again (U_2 or S_2) at exactly 120 seconds (60 seconds after first reading).

III- URINE TECHNIQUE

Follow the above technique (I or II) diluting the sample properly with water or saline. Calculate the results, multiplying by the dilution factor used.

CALCULATIONS

$$\text{Urea (g/l)} = f \times (U_1 - U_2) \qquad f = \frac{0.60 \text{ g/l}}{(S_1 - S_2)}$$

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known urea concentration.

REFERENCE VALUES

Serum or plasma

0.10 - 0.50 g/l as urea (4.7-23.4 mg/dl as BUN)

This range was obtained from sera of 120 fasting individuals from both sexes, with ages ranging from 20 and 45 years, living in or near Rosario (Argentina), with no symptoms of renal dysfunction or other apparent disease.

Urine

Normally, urea elimination shows great variations depending on diet. On average and with an ordinary mixed diet, 30 g are excreted in 24 hours, with oscillations ranging from 20 g to 40 g.

In the literature (Tietz, N.W.) the following reference value range is mentioned:
Serum or plasma: 13 - 43 mg/dl (2,1 - 7,1 mmol/l)
Urine: 26 - 43 g/24 hs (0,43 - 0,72 mol/24 horas)

It is recommended that each laboratory establishes its own reference values.

UNITS CONVERSION

Urea (g/l) x 46.7 = BUN (mg/dl)
Urea (mg/dl) x 0.1665 = Urea (mmol/l)
Urea (mg/dl) x 0.467 = BUN (mg/dl)
BUN (mg/dl) x 2.14 = Urea (mg/dl)
Urea (g/24 hs) x 0.0167 = Urea (mol/24 hs)

To convert Urea values (in g/l) to BUN values (in mg/dl) a conversion factor must be used.

$$\text{Conversion factor} = \frac{1}{2.14} \times \frac{1000}{10} = 46.7$$

where:
1/2.14 = conversion factor between urea and blood urea nitrogen (BUN)
1000 = conversion factor between gram and milligram
1/10= conversion factor between liter and deciliter

Example: Urea 0.50 g/l x 46.7 = BUN 23.4 mg/dl

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
In order to preserve the reagent's integrity, perfectly clean and dry volumetric material should be used.

PERFORMANCE

The assay were performed in an Express plus analyzer (*)
a) Reproducibility: when 20 replicates from the same sample were simultaneously assayed, the following results were obtained:

Intra-assay

Level	S.D.	C.V.
0.283 g/l	± 0.0057 g/l	2.01 %
1.13 g/l	± 0.0136 g/l	1.20 %

Inter-assay

Level	S.D.	C.V.
0.28 g/l	± 0.0066 g/l	2.36 %
1.13 g/l	± 0.0148 g/l	1.31 %

b) Sensitivity: the analytical sensitivity of **Urea UV cinética AA líquida** is 0.071 g/l (7.1 mg/dl) of urea or 3.32 mg/dl BUN and the detection limit is 0.0383 g/l (3.83 mg/dl) of urea or 1.79 mg/dl BUN.
c) Linearity: reaction is linear up to 3 g/l (300 mg/dl) as urea and up to 140 mg/dl BUN. For higher values dilute original sample 1:2 with distilled water and repeat assay. Correct

calculations multiplying the result by the dilution factor used.
d) Correlation: urea levels of 158 specimens were determined using the Wiener lab's **Urea UV cinética AA líquida** kit and a commercial kit based on same principle. The correlation coefficient was:
r = 0.9995, slope b = 1.0093 and intercept a = - 0.0985.

PARAMETERS FOR AUTOANALYZERS
For programming instructions check the user's manual of the autoanalyzer in use. For calibration, it must be used Wiener lab.'s **Calibrador A plus**.

WIENER LAB. PROVIDES
- 225 ml (3 x 60 ml Reagent A + 3 x 15 ml Reagent B), non-included Standard (Cat. Nº 1009319).
- 300 ml (4 x 60 ml Reagent A + 1 x 60 ml Reagent B), non-included Standard (Cat. Nº 1009634).
- 400 ml (8 x 40 ml Reagent A + 4 x 20 ml Reagent B), non-included Standard (Cat. Nº 1009275).
- 500 ml (4 x 100 ml Reagent A + 4 x 25 ml Reagent B), included Standard (Cat. Nº 1810324).
- 1000 ml (4 x 200 ml Reagent A + 1 x 200 ml Reagent B), included Standard (Cat. Nº 1810328).

REFERENCES

- Searcy, R.L. - "Diagnostic Biochemistry" McGraw-Hill, New York, NY 1969.
- Talke, H.; Schubert, G.E. - Klin Wochschr 43:174, 1965.
- Tiffany, T.O.; Jansen, J.M.; Burtis, C.A.; Overton, J.B.; Scott, C.D. - Clin. Chem. 18:829, 1972.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 3rd ed., 1990.
- Tietz Fundamentals of clinical chemistry - Burtis, C., Ashwood, E. (5th Edition) WB Saunders, 2001.

SYMBOLS
The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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