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:

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

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

PROVIDED REAGENTS

S. Standard: 0.60 g/l urea solution (28.04 mg/dl BUN).
A. Reagent A: vials containing 2-Oxoglutarate, NADH, Urease and Glutamate Dehydrogenase (GlDH).
B. Reagent B: Goods buffer solution pH 7.8 ± 0.1.

Final concentrations

2-Oxoglutarate	7.5 mmol/l
NADH	0.28 mmol/l
Urease (Jack bean)	≥ 4000 U/l
GlDH (microbial)	≥ 400 U/l
Goods	100 mmol/l

INSTRUCTIONS FOR USE

Standard: ready to use.
Working Reagent:
- 4 x 50 ml: dissolve the content of a vial of Reagent A into a Reagent B bottle. Rinse the vial several times with Reagent B. Mix gently by inversion until complete dissolution. Avoid foaming. Date.
- 10 x 20 ml: dissolve the content of a vial of Reagent A with 20 ml Reagent B. Mix gently by inversion until complete dissolution. Avoid foaming. Date.

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

INSTABILITY OR DETERIORATION OF REAGENTS

When spectrophotometer has been set to zero with distilled water, Working Reagent Absorbance readings lower than 1.000 O.D. (at 340 nm) indicate 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. Do not use ammonium heparin.
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
- Wavelength: 340 nm (Hg 334 or 366)
- Reaction temperature: 37ºC
- Reaction time: 2 minutes
- Sample volume: 10 ul
- Working Reagent volume: 1 ml
- Final reaction volume: 1.01 ml

Sample and Reagent volumes may be proportionally altered

in order to meet the requirements of different spectrophotometers.

PROCEDURE

Bring the reagents to working temperature before sample addition. Set spectrophotometer to zero O.D. with distilled water.

In a cuvette at the selected temperature, place:

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

Mix immediately 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). Determine the absorbance difference (ΔA). Use this difference for calculations.

URINE TECHNIQUE

Follow the above technique 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 precision

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 precision

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** 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** 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 manual of the autoanalyzer in use. For calibration, it must be used Wiener lab.'s **Calibrador A plus**.

WIENER LAB. PROVIDES

- 10 x 20 ml (Cat. Nº 1810322).
- 4 x 50 ml (Cat. Nº 1810323).

REFERENCES

- Searcy, R.L. - "Diagnostic Biochemistry" McGraw-Hill, New York, NY 1969.

** TM Ciba Corning Diagnostics*

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- 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, 4th ed., 2001.
- Faulkner, W.R.; King, J.W. - "Fundamentals of Clinical Chemistry". Tietz NW (Ed) W.B. Saunders Co. Philadelphia Chap 12:718, 1970.
- 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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