ISE S.r.l. Urea UV – Instructions for use

CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CE IVD

R1: 2x 18 mL R2: 2x 4,5 mL **R3330000026** REF

Diagnostic reagent for quantitative in vitro determination of urea in human serum, plasma or urine on photometric systems

TEST PARAMETERS

Method:	UV, 2 Point Kinetic (fixed time), decreasing reaction, GLDH
Wavelength	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	25 °C, 30 °C or 37 °C
Sample:	Serum, plasma, urine
Linearity:	up to 300 mg/dL (50 mmol/L) on Hitachi 911
Sensitivity:	The lower limit of detection is 2 mg/dL (0.3 mmol/L)

SUMMARY [1,2]

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Parallel determination of uea and creatinine is performed to differentiate between pre-renl and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemias, for example caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent. In renal diseases urea concentrations are elevated when the glomerular filtration rate is markedly reduced and when the protein intake is higher than 200 g/day.

TEST PRINCIPLE

$$Urea + 2H_2O \xleftarrow{Urease} 2\,NH_4^+ + HCO_3^{2-}$$

$$NH_4^+ + 2\text{-Oxoglutarate} + NADH \xleftarrow{GLDH} L\text{-Glutamate} + NAD^+ + H_2O$$

Decrease in absorbance, resulting from the GLDH-reaction, is proportional to the concentration of Urea in the sample.

ABBREVIATIONS

NAD	=	Nicotinamide Adenine Dinucleotide
NADH	=	reduced NAD
GLDH	=	Glutamate Dehydrogenase
ADP	=	Adenosine diphosphate

REAGENT COMPOSITION

COMPONENTS	CONCENTRATIONS
Reagent 1	
Tris Buffer, pH 7.8	150 mmol/L
2-Oxoglutarate	9 mmol/L
ADP	0.75 mmol/L
Urease	≥ 7 KU/L
GLDH (Glutamate dehydrogenase)	≥ 1 KU/L
Reagent 2	
NADH	1.3 mmol/L

REAGENT PREPARATION

Substrate Start:
The reagents are ready to use.
Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2 (= Working Reagent).
Leave the working reagent for at least 30 min. at 15 – 25 °C before use.

REAGENT STABILITY AND STORAGE

Conditions: Protect from light. Close immediately after use. Do not freeze the reagents! Avoid contamination.

Substrate Start:
Stability: at 2 – 8°C up to the expiration date

Sample Start (Working Reagent):

Stability: at 15 – 25 °C 5 days
at 2 – 8 °C 4 weeks
Protect the working reagent from light!

SAMPLE PREPARATION

Urine: Dilute urine 1 + 50 with dist. Water and multiply results by 51.
The Urine controls must be prediluted the same way as patient samples.

SAMPLE STABILITY AND STORAGE [4]

Do not use ammonium heparin plasma!

serum or plasma:	at 20 – 25 °C	7 days
	at 4 – 8 °C	7 days
	at - 20 °C	1 year
urine:	at 20 – 25 °C	2 days
	at 4 – 8 °C	7 days
	at - 20 °C	1 month

Freeze only once! Discard contaminated specimens.

STANDARD

(not included in the kits – has to be ordered separately)
Concentration 50 mg/dL (8.33 mmol/L)
Storage: 2 – 25 °C
Stability: up to the expiration date
Close immediately after use! Avoid contamination!
Protect from light.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.
Reagent start

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard/Calibrator	-	10 µl	-
Mix. Incubate for up to 0-5 minutes, then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix, incubate for 60 sec. at 25/30 °C or 30 – 40 sec. at 37 °C and measure absorbance A1 against reagent blank. Incubate for exactly 60 sec. and measure absorbance A2 against reagent blank. Calculate ΔA /min = A1 – A2.			

Sample start

Pipette into test tubes	Blank	Std./Cal.	Sample
Working Reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Stdandard/Calibrator	-	10 µl	-
Mix, incubate for 60 sec. at 25/30 °C or 30 – 40 sec. at 37 °C and measure absorbance A1 against reagent blank. Incubate for exactly 60 sec. and measure absorbance A2 against reagent blank. Calculate ΔA/min = A1 – A2.			

Note:
The method is optimized for 2-point kinetic measurement. It is mandatory to incubate all samples and the reagent blank **strictly** for the same time intervals. This method is therefore recommended for automated test procedure on automatic analysers.

CALCULATION

serum/plasma:

$$\text{Urea (mg/dl)} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Std/Cal}} \times \text{Conc. Std/Cal (mg/dl)}$$

urine:

$$\text{Urea (mg/dl)} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Std/Cal}} \times \text{Conc. Std/Cal (mg/dl)} \times 101$$

UNIT CONVERSION

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]
(BUN: Blood Urea Nitrogen)

REFERENCE RANGE *

In serum / plasma [1]:

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Adults:	[mg/dL]	[mmol/L]
Global	17 – 43	2.8 – 7.2
Women < 50 years	15 – 40	2.6 – 6.7
Women > 50 years	21 – 43	3.5 – 7.2
Men < 50 years	19 – 44	3.2 – 7.3
Men > 50 years	18 – 55	3.0 – 9.2
Children:		
1 – 3 years	11 – 36	1.8 – 6.0
4 – 13 years	15 – 36	2.5 – 6.0
14 – 19 years	18 – 45	2.9 – 7.5

BUN in serum / plasma:

Adults:	[mg/dL]	[mmol/L]
Global	7.94 – 20.1	2.8 – 7.2
Women < 50 years	7.01 – 18.7	2.6 – 6.7
Women > 50 years	9.81 – 20.1	3.5 – 7.2
Men < 50 years	8.87 – 20.5	3.2 – 7.3
Men > 50 years	8.41 – 25.7	3.0 – 9.2
Children:		
1 – 3 years	5.14 – 16.8	1.8 – 6.0
4 – 13 years	7.01 – 16.8	2.5 – 6.0
14 – 19 years	8.41 – 21.0	2.9 – 7.5

Urea/Creatinine ratio in serum [1]:
25 – 40 [(mmol/L)/(mmol/L)]
20 – 35 [(mg/dL)/(mg/dL)]
Urea in urine [2]:
26 – 43 g/24h (0.43 – 0.72 mol/24h)

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY / MEASURING RANGE

The test has been developed to determine urea concentrations within a measuring range from 2 – 300 mg/dL (0.3 – 50 mmol/L) in serum/plasma respectively up to 30 g/dL (5 mol/L). If values exceed this range the samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.
Note: Linearity may be different on different analysers and with different instrument applications.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 2 mg/dL (0.3 mmol/L).

PRECISION (at 37°C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	21.3	0.50	2.33
Sample 2	35.3	0.82	2.33
Sample 3	141	1.52	1.08

Inter-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	20.3	0.58	2.88
Sample 2	48.3	1.12	2.32
Sample 3	152	1.38	0.91

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
triglycerides	2000 mg/dL

Ammonium ions interfere, therefore do not use ammonium herparin as anticoagulant for collection of plasma!

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison between this Urea (y) and a commercially available test (x) using 68 samples gave following results: y = 0.99 x + 1.06 mg/dL; r= 0.999.

CALIBRATION

The assay requires the use of a uric acid standard or calibrator.

QUALITY CONTROL

All controls with urea values determined by this method can be used.
Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 1 contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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4. Guder WG, Zawta Be et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 48-9.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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