ASSEL S.r.l.
Via E. Barsanti 13/A – 00012 Guidonia (Rm)
Tel.: +39 0774 357492
Fax: +39 0774 372179
e-mail: info@asselitaly.eu

AZIENDA CERTIFICATA
UNI EN ISO 9001:2008
UNI EN ISO 13485:2004
CERTIFIED COMPANY

UREA UV SL

Kinetic enzymatic method

IVD CE In conformity with Directive 98/79 CE

000271 Rev. 6 of 06/04/2010

REF		
	ASR01143	**2 x 80 + 1 x 40 ml + STD**
	ASR01144	**4 x 80 + 1 x 80 ml + STD**
	ASKIT2602	**4 x 36 + 4 x 9 ml**

• Principle

This is an enzymatic determination which follow this principle:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + C0_2$$

$$2NH_4 + 2\ \alpha\text{-Chetoglutarate} + 2NADH \xrightarrow{\text{GLDH}} \text{2L-glutammate} + 2NAD^+ + 2H_2O$$

Decreasing of NADH concentration is proportional to urea concentration in the sample.

• Sample

Serum, plasma with heparin.
Urine: urine must be diluted 1:100 with physiological solution.
Do not use anticoagulant containing fluoride or ammonium salts.
Urea in serum or plasma is reported stable up to 62 hours at 2 – 8°C, and 8 hours at room temperature.
Shake and bring the samples at room temperature before using.

• Expected value

Serum and plasma	10 – 50 mg/dl (1.7 – 8.3 mmol/l)
Morning urine	847 – 2967 mg/dl (141 – 494 mmol/l)
24 h urine	10 – 35 g / 24 h (170 – 580 mmol/24 h) correspondent to 670–2300 mg/dl (110-390 mmol/l*) * founded on urine volume 1.5 l / 24 h

Consider the above mentioned values as a reference.
It is strongly recommended that each laboratory establishes its own normal range according to its geographic area.

• Kit composition and possible risk classification

REAGENT (A)	
Good's Buffer	100 mmol/l
ADP	1 mmol/l
Alfa-Chetoglutarato	9 mmol/l
Urease	8100 U/l
GLDH	1350 U/l
REAGENT (B)	
NADH	1.5 mmol/l
STANDARD (C)	
Urea	50 mg/dl (8.325 mmol/l)

The kit doesn't contain substance or prepared classified as dangerous according to the currently legislation.

• Package: collection and storage

Store in refrigerator (2 – 8°C).
Stable until the expiration date reported on the package.
After unsealing, it is advised to close up the bottle immediately in order to avoid evaporation, direct light exposure and bacteric contamination.

• Reagent preparation and stability

Reagents must be at room temperature (15 – 25°C) before using.
The Reagents are limpid and colourless.
Monoreagent procedure:
Add 1 part of reagent (B) to 4 parts of reagent (A).
Reagent (A+B) is stable up to 5 days at room temperature and 4 weeks if stored in refrigerator.
Bi-reagent procedure:
Ready to use liquid reagents.

• Precautions and warning

Whenever agents infettantis, chemical reagents, reagents of human/animal origin, blood or other biological liquids are manipulated, it is advisable to follow the most common recommendations and take all the necessary hygienic precautions as the monouse gloves.

• Waste disposal

Please consult local regulations for a correct waste disposal.

• Operative method

Wavelength:	340nm (334-365)
Cuvette:	1cm l
Temperature:	37°C
Zero operation:	Against distilled water
Assay type:	Decreasing kinetic
Sample/Reagent ratio:	1/100

Mono reagent procedure:

Pipetting in tubes:	SAMPLE	STANDARD
Reagent (A + B)	1000 µl	1000 µl
Sample	10 µl	
Standard		10 µl

Bi-reagent procedure:

Pipetting in tubes:	SAMPLE	STANDARD
Reagent (A)	1000 µl	1000 µl
Sample	10 µl	
Standard		10 µl

Mix, incubate for 60''

Reagent (B)	250 µl	250 µl

In both procedures proceed as follows:
Adjust the instrument to zero with distilled water.
Mix, transfer in cuvette and incubate for 30'' at 37°C (T.0"); read sample and standard extinction at time 0'' and after 1'.
Calculate ΔE/min. a 340nm.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit.
Using automated instrumentations, calibration with watery standard may cause a systematic error.
Human serum calibrator REF. ASR02031 is suggested.

• Calculation

$$\text{UREA mg/dl} = \frac{\Delta E/\text{min Sample}}{\Delta E/\text{min Standard}} \times 50\ (\text{Value Standard})$$

Standard Value 50mg/dl = 8.325mmol/l.
Urea values can be converted in BUN by multiplying them by the factor 0.466. Take into account dilution factor for urine.

• Performance

A. METHOD PERFORMANCE

Measure interval / linearity:	5.5 – 300 mg/dl
Detection limit (2DS):	5.5 mg/dl
Sensitivity:	1 mg/dl = 1.33 ΔE/min

B. INTRA-ASSAY PRECISION: n=20

	Mean	C.V.(%)
Low control	M = 28 mg/dl	2.8 %
Medium control	M = 51 mg/dl	3.3 %
High control	M = 159 mg/dl	3.9 %

C. INTER-ASSAY PRECISION: n=20

	Mean	C.V.(%)
Low control	M = 29 mg/dl	3.2 %
Medium control	M = 50 mg/dl	3.8 %
High control	M = 158 mg/dl	4.4 %

D. CORRELATION BETWEEN METHODS

This method compared with a correspondent one from the competition, has given the following results:
N = 95 r = 0.999 y = 1.02 x – 0.78

E. INTERFERENCE (in accordance with recommendations SFBC)
The interferences are neglected up to:

Bilirubine	60 mg/dl	Triglicerides	1000 mg/dl
Hemoglobin	0.5 g/dl	Glucose	500 mg/dl
Ascorbic Acid	23 mg/dl	Metildopa	50 mg/dl

• Limitations

Samples with high ammonia concentration could cause high urea results.

• Quality Control

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology. For this purpose the use of test serum REF. ASR02010 (Normal level) and REF. ASR02020 (Pathologic level) is suggested.

• Bibliography

1) Bretaudiè, J.P., et al. Clin. Chem., 22/10, 1614, (1976).
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3) Mackay EM, Mackay LL. J Clin Invest 1927; 4:295.
4) Krieg M e al., J Clin Chem Clin Biochem 1986; 24:863.
5) Colombo J-P (ed) Klinish-chemische Urindiagnostick. Rotkreuz: LABOLIFE-Verlagsgesellschaft, 1994:18

UREA UV SL

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REF

ASR01143	**2x80 + 1x40 ml + STD**
ASR01144	**4x80 + 1x80 ml + STD**
ASKIT2602	**4x36 + 4x9 ml**

		ELLIPSE rev.0 of 28/06/10	LIASYS rev.0 of 28/06/10
Description:		UREA UV	UREA UV
Unit:		mg/dl	mg/dl
Decimals:		0	0
LIS Code:		UREA	UREA
Unit Factor:		1.0	1.0
Slope:		1.00	1.00
Intercept:		0.00	0.00
Reaction Type:		Fixed Time	Fixed Time
Direction:		Down	Down
E.P Limit:			
Depl.Limit:		0.6000	0.6000
First Limit:		1.0000	1.0000
Linear Factor:		1.00	1.00
Fit:			
RBL Replicates:		X1	X1
RBL Max CV%:		10	10
RBL Min (abs):		0.9000	0.9000
Max (abs):		1.8000	1.8000
Lin. Lim. Low:		0	0
High:		300	300
Rerun when over:		*	*
Calculation Model:		Standard	Standard
Factor:		1.00	1.00
Sample Blank:		No	No
Reference Range		*	*
Parameters			
Predilut	Times (sec):		
	Dil./Reag Code:		
	Lot Number:		
	Ratio/Vol (ul):	1/1	1/1
C. + R.1	Times (sec):	0	0
	Dil./Reag Code:	UREA	UREA
	Lot Number:		
	Ratio/Vol (ul):	220	300
	Rinse (ul):	0	0
	Sample (ul):	2	3
Reag 2	Times (sec):		
	Dil./Reag Code:		
	Lot Number:		
	Ratio/Vol (ul):		
	Rinse (ul):		
	Sample (ul):		
Reag 3	Times (sec):		
	Dil./Reag Code:		
	Lot Number:		
	Ratio/Vol (ul):		
	Rinse (ul):		
	Sample (ul):		
Wash			
Incubation		18	26
Read		78 kinetic	72 kinetic
Filter 1 (nm):		340	340
Filter 2 (nm):		None	None
Bichr. Factor:		1.00	1.00
RBL Stability (days):		1	1
Calibration Stab. (days):		7	7
Dinamic Controls (min.):		*	*

* user-defined

• **Performance ELLIPSE**

METHOD PERFORMANCE

Linearity**:** 319,14 mg/dl

Sensitivity: 1 mg/dl = 0.0022A

STABILITY ON BOARD:

Stable two weeks if reagent is kept refrigerated and capped (after working session)

INTRA-ASSAY PRECISION: n=40

	Media	DS	CV
Normal control	45,28	0,61	1,35
Abnormal control	151,95	2,17	1,43

INTER-ASSAY PRECISION: n=40

	Media	DS	CV
Normal control	45,28	1,23	2,72
Abnormal control	151,95	3,60	2,37

CORRELATION BETWEEN METHODS

This method compared with a correspondent one from the competition, has given the following results:

N = 135 r = 0,99 y = 1,1926x - 4,977

• **Performance LIASYS**

METHOD PERFORMANCE

Linearity**:** 317,26 mg/dl

Sensitivity: 1 mg/dl = 0.0022A

STABILITY ON BOARD:

Stable two weeks in refrigerated compartment and capped overnight

INTRA-ASSAY PRECISION: n=40

	Media	DS	CV
Normal control	44,95	0,92	2,05
Abnormal control	150,70	2,12	1,41

INTER-ASSAY PRECISION: n=40

	Media	DS	CV
Normal control	44,95	1,31	2,91
Abnormal control	150,70	5,06	3,36

CORRELATION BETWEEN METHODS

This method compared with a correspondent one from the competition, has given the following results:

N = 135 r = 0.99 y = 1,1539x - 3,8749