UREA U.V.

Enzymatic and colourimetric determination of Urea in biological liquids

TEST SUMMARY

The Urea is being hydrolized by means of Urease to Ammonia, which reacts in presence of Glutamate dehydrogenase, with 2-oxoglutarate and NADH to form glutamate and NAD^+.
The decrease of absorbance is measured at 340 nm.

SAMPLES

Serum, plasma (avoid ammonium heparinate).
Urine diluted 1:100 with deionized water.
Urea is stable 3 days at 2-8°C.

REAGENTS

Reagent A:	Tris pH 7.60 115 mM, ADP 1.2 mM, NADH 0.25 mM, Urease ≥ 8 KU/lt, GLDH ≥ 800U/lt, stabilizers.
Reagent B:	2-oxoglutarate Sodium 7.5 mM, stabilizers.
Standard:	Urea 50 mg/dl; stabilizers and preservatives.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solutions.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GPL) guidelines.

REAGENTS PREPARATION

Add 20 ml of reagent B to a bottle of reagent A.
Stability: 60 days at 2-8°C.
Keep at room temperature before use.

TEST PROCEDURE (STARTER SAMPLE)

Kind of analysis:	Fixed time (decreasing)
Reading time:	30, 90 seconds
Delay:	30 seconds
Wavelenght:	340 nm
Temperature:	37°C
Lightpath:	1 cm
Zero:	Blank reagent

REAGENTS	BLANK	STANDARD	SAMPLE
Working reagent	1 ml	1 ml	1 ml
Incubate at 37 °C for 5 minutes			
Distilled water Standard Sample	10 µl -- --	-- 10 µl --	-- -- 10 µl
Record the absorbance after 30 seconds (A1) at 340 nm, record again after exactly 60 seconds (A2).			

CALCULATION

Serum/Plasma Urea (mg/dl)

$$[A_2 - A_1\,(_{sample})] / [A_2 - A_1\,(_{standard})] \times 50$$

(standard value)

Random urine Urea (mg/dl)

$$[A_2 - A_1\,(_{sample})] / [A_2 - A_1\,(_{standard})] \times 50 \times 100$$

(standard value and dilution)

24 hours urine Urea (g/24h)

$$\frac{[A_2 - A_1\,(_{sample})] / [A_2 - A_1\,(_{standard})] \times 50 \times 100 \times \text{dl urine}}{1000}$$

(standard value, dilution factor and diuresis in decilitres)

EXPECTED VALUES

Adults	10 – 50 mg/dl	(1.7 – 8.3 mmol/l)
Urine	20 – 35 g/24 h	(332 – 580 mmol/24 h)

Each laboratory should establish appropriate reference intervals related to its population.

NOTE

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

QN 0050 CH **10 x 5 ml**
Control Sera normal values

QP 0050 CH **10 x 5 ml**
Control Sera pathological values

TEST PERFORMANCE

Precision

Intra-assay (n = 30)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	41.23	0.7738	1.88
Sample 2	129.7	0.7497	0.58

Inter-assay (n = 30)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	40.56	1.3308	3.28
Sample 2	130.30	1.2077	0.93

Sensitivity/Limit of detection
The method is able to discriminate until 1 mg/dl.

Linearity
The method is linear up to 300 mg/dl.
If the values are exceeded, it is suggested to dilute the sample 1+9 with saline and to repeat the test, multiplying the results by10.

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 100 samples:

Urea LTA = x
Urea competitor = y
n = 31

y = 0,98577x + 0.38516 mg/dl r = 0,9795

Interferences
No interference was observed by the presence of:

haemoglobin	≤ 500 mg/dl
bilirubin	≤ 28 mg/dl
lipids	≤ 600 mg/dl

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC02301	(400 TESTS)	
Reagent A	4 x 80 ml	(liquid)
Reagent B	2 x 40 ml	(liquid)
Standard	1 x 5 ml	(liquid)

REFERENCES

Falke, H.N. Schubert, G.E. Klin. Wschr.42 (1965).
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashood (1994).
HU Bergmeyer – Methods of enzymatic analysis, (1987).

MANUFACTURER

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SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

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