Urea

Liquid Reagent
Enzymatic. Kinetic Method
Store at 2-8ºC

PRINCIPLE

Urea is hydrolyzed by water and Urease into ammonia and carbondioxide. The ammonia produced is further acted with ketoglutarate and NADH in the presence of GLDH to reproduce glutamate and NAD according to the following reaction :

$$\text{Urea} + H_2o \xrightarrow{\text{Urease}} 2\,NH_3 + Co_2$$

$$2NH_4^+ + 2\,\alpha\ \text{Ketoglutarate} \xrightarrow{\text{GLDH}} \gamma\ \text{Glutamate} + 2\,NAD^+ + H_2o$$

REFERENCE VALUES

Serum , plasma	18-45	mg/dl
Urine	20 – 35	g/24hrs
	338-538	mmol/24 h

Theses ranges are given for orientation only, each laboratory should establish its own normal ranges.

SAMPLES

Serum, heparin plasma.
Urine diluted 1/100 with distilled water, (Do not use anticoagulants containing fluoride or ammonium ions).

REAGENTS

R1 + R2 Concentration in the test :

Tris buffer	70 mmol/l
α-Ketoglutarate	≥ 5 mmol/l
GLDH	≥ 6 UI/ml
NADH	0.35 mmol/l
Urease	≥ 4 UI/ml

R_3:

Standard	50 mg/dl

PREPARATION OF WORKING REAGENT

Reagent R1 and R2 are ready to use. If a monoreagent procedure is preferred then the reagent must be mixed in the ration 4 parts of R1 to 1 part of R2. The working reagent is stable for one month at 2-8ºC

PROCEDURE

Wavelength	340 nm
Temperature	25ºC/30ºC /37ºC
Zero adjustment	Air or distilled water
Cuvette	1 cm light path
Method	Kinetic - Decreasing

If the absorbance of the working reagent is lower than 1.1 at 334nm the reagent can not be used.

	Standard	Sample
Standard	10 µl	-
Sample	-	10µl
Working reagent	1ml	1ml

Mix well and read the optical density after 30 seconds ($O.D_1$), and after 90 seconds ($O.D_2$).

CALCULATION

$$\frac{O.D1 - O.D2\ \text{Sample}}{O.D1 - O.D2\ \text{Standard}} \times \text{Standard concentration}$$

LINEARITY

Up to 200 mg/dl or 36 mmol/l.

SENSITIVITY

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a chang of 0.001 abs units/min is equivalent to 0.009mmol/l Urea concentration at 334nm.

SPECIFICATION

Bilirubin 0.5g/l, lipid 10g/l, glucose 10g/l and ascorbic acid 0.5g/l do not interfere with the assay up to the given levels

NOTES

- Do not use hemolized serum.
- Urea in sample is stable for 7 days at 2-8ºC.

PRESENTATION

3 X 60 ml Cat No 3001 180 Tests

Bibliography

- Levitsky A., Analy. Biochem.23. 335(1970).
- Chaney. AL. Clin. Chem 8. 130 (1962).

The following symbols are used on labels

IVD	**For in vitro diagnostic use**
	Use day (last day of the month)
	Temperature limitation
LOT	**Batch code**
REF	**Code**