Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 14611 R1 3 x 40 ml
For 180 tests R2 3 x 20 ml

Urea

Fully enzymatic UV method

Liquid Reagents

Test Principle

kinetic determination of urea according to the follwing reactions:

$$\text{Urea} + H_2O \xrightleftharpoons{\text{Urease}} 2\ NH_3 + CO_2$$

$$NH_4^+ + \alpha\text{-Ketoglutarate} + \text{NADH} \xrightleftharpoons{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

Urea is hydrolyzed by urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction resulting in a decrease in absorbance at 340 nm that is directly proportional to the urea concentration in the sample.

Concentrations in the test

Reagent R1		
Tris-Buffer	20	mmol/L
α-ketoglutarate	8	mmol/L
NADH	325	µmol/L
Detergent, preservative.		
Reagent R2		
Tris-Buffer	20	mmol/L
ADP	10	mmol/L
Urease	≥ 30	KU/L
GLDH (Glutamate-Dehydrogenase)	≥ 1	KU/L
Detergent, preservative.		
Standard : The Concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R1: *liquid*.
Reagent R2: *liquid*.

All reagents are stable up to expiry date given on label when stored at +2 ➔ +8°C.

Working Reagent:
Mix 2 volumes of bottle R1 with 1 volume of bottle R2.
Stability: 2 months at 2 - 8 °C.

Note: Don't use if the working reagent has an absorbance less than 1.0 at 340 nm.

Specimen collection and handling

1. Serum is the recommended sample.
2. EDTA, citrate, Na-Heparin, Na-Fluoride or oxalate anticoagulants could be used.
3. Anticoagulants containing ammonium or fluoride salts should not be used.
4. All material coming in contact with the sample must be free of ammonia and heavy metals.
5. Urea in serum is reported stable for seventy-two hours refrigerated at +2 to +8 °C. Unrefrigerated sera should be used within eight hours.
6. Fresh urine: dilute urine (1+20) with double distilled water and multiply result by 21.

Calibrator / Standard

MediCal U Cat. No. 15011
Urea STD. Cat. No. 16201

Quality control

Meditrol N Cat .No 15171
Meditrol P Cat .No 15181

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – decrease

Assay : incubate reagent at 37 °C before use:

	Calibrator/ Standard	Sample
Calibrator / Standard	10 µl	--
Sample	--	10 µl
Working Reagent	1000 µl	1000 µl
Mix, incubate 1 min. at 37°C.Read change in absorbance per 1 min. for 3 min. Determine the mean absorbance change per 1 min. (ΔA/min).		

Calculation

Serum:

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

Urine:

$$\text{Conc.}_{\text{Urea}}\ (\text{mg/dl}) = \frac{\Delta A_{/\text{min. Sample (Dilute)}}}{\Delta A_{/\text{min Cal./STD.}}} \times \text{Conc.}_{\text{Cal./STD.}}(\text{mg/dl}) \times \text{Dilution Factor}$$

$$\text{Conc.}_{\text{Urea Urine 24/hr.}} = \frac{\text{Urea mg/dl (Urine)} \times (\text{vol./ ml})_{\text{Urine 24 /hr.}}}{100\ 000}\ \text{g/24 hr.}$$

$$\text{mmol/L} \xrightleftharpoons[0.166 \times]{\times 6} \text{mg/dl}$$

Linearity

Up to 250 mg/dl (41.5 mmol/L).
If result is higher, repeat test using sample diluted (1+1) with sodium chloride solution (0.9 %) and multiply result by 2.

Interferences

1. Urease action is inhibited by fluoride.
2. Bilirubin higher than 20 mg/dl, hemoglobin higher than 500 mg/dl, and ascorbic acid higher than 200 mg/dl have negligible interference in this assay.

Precautions

Reagents contain sodium azide as preservative. Avoid any contact with skin and mucous membranes. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.

Reference range

Serum

New born	< 42	mg/dl
≤ 6 mth.	< 42	mg/dl
≥ 7 mth.	< 48	mg/dl
Adults	< 50	mg/dl

Urine

1st morning urine	900 - 3000	mg/dl
Urine / 24 hr.	10 - 35	g/24 hr.

References

1. Gutmann, I. and H.U. Bergmeyer in H.U. Bergmeyer: Methoden der enzym. Analyse, 3. Aufl. Bd. II, Verlag. Chemie Weinheim, 1974, S. 1842.
2. MacKay, E.M. u. L.L. MacKay, J. Clin. Invest. 4 (1927) 295.
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4. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.