Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 14601
For 200 test

R1	4	x	50	ml
R2	1	x	4	ml
R3	1	x	40	ml

Urea

Berthelot method, Enzymatic colorimetric test

Liquid Reagents

Principle

Enzymatic determination of urea according to the following reactions:

$$\text{Urea} \xrightarrow{\textit{Urease}} 2\ NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} + NaOCl \xrightarrow[OH^-]{\textit{nitroprusside}} \text{Dicarboxyl indophenol}$$

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonium ions react with salicylate and hybochlorite to form a green colored indophenol (2.2-dicarboxyl-indophenol). The intensity of this dye is proportional to the concentration of urea in the sample.

Concentrations in the test

Reagent R1		
Phosphate buffer, (pH = 7.0)	50	mmol/L
Sodium salicylate	62	mmol/L
Sodium nitroprusside	3.5	mmol/L
EDTA	1.2	mmol/L
Reagent R2		
Urease	≥ 50	KU/L
Reagent R3		
Sodium hydroxide	900	mmol/L
Sodium hypochlorite	3.75	mmol/L
Standard : The Concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *liquid.*
Reagent R3: *liquid, ready to use.*

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

Working Reagent:
Add **EXACTLY** 1 ml R2 to a bottle of 50 ml R1.
(be sure that you pipette all 1 ml R2)
Mix gently without foaming for 15 minute before use.
keep the working reagents in the same dark bottle R1.
Stability: 1 months at 2 - 8 °C.

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 U Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 578 nm
Spectrophotometer	580 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator/ Standard	Sample
Dist. water	10 µl	-	-
Calibrator / Standard	-	10 µl	-
Sample	-	-	10 µl
Working Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37°C or 10 min. at 20 – 25 °C.			
Reagent R3	200 µl	200 µl	200 µl
Mix, incubate for at least 7 min. at 37°C or 10 min. at 20 - 25°C. Read absorbance (A). The final color is stable for 1 hour.			

Calculation

Serum:

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

Urine:

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

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

$$\text{mmol/L} \underset{0.166 \times}{\overset{\times\ 6}{\rightleftharpoons}} \text{mg/dl}$$

Urea Berthelot method, Enzymatic colorimetric test

Linearity

Up to 200 mg/dl (33.3 mmol/L).
If the result exceeds 200 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. Urease action is inhibited by fluoride and heavy metals.
2. Ammonia contamination of glassware, reagents or atmosphere is the main source of error.
3. Bilirubin higher than 20 mg/dl , hemoglobin higher than 500 mg/dl and triglycerides higher than 800 mg/dl have been found to exhibit negligible interference in this assay.
4. For a comprehensive review of drug interference see Young, et al.

Precautions

1. Reagent R3 is an alkaline solution. Avoid contact with skin. Flush with plenty of water if contact occurs . Don't pipette by mouth.
2. Avoid contamination with ammonium.
3. Don't expose the reaction medium to direct strong light.
4. Reagent R1 contain sodium azide as a preservative. 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. Krupp, M.A., et. al, 20th Ed., Lange Medical Publications, Los Altos, CA, p.216 (1982).
2. Kaplan, A. And Teng, L.L. in Selected Methods of Clinical Chemistry, Vol. 9, Ed. By W.R. Faulkner and S. Mietes, AACC, Washington, pp 357 - 363 (1982).
3. Tietz, NW., textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia, p. 1270 - 1271 (1986).
4. Young, D.S., et al, Clin. Chem. 21:1D (1975).
5. Friedman, R.B. et al, Clin. Chem., 26: 1D (1975)