UREA (Kin)
(UV GLDH Method)

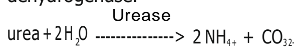
CHEMCount

INTRODUCTION

Urea is a product of amino acids catabolism. It is produced in liver and excreted in urine. Urea in the blood is reported as the blood urea nitrogen (BUN). Increased urea concentration in the serum, called uremia, is observed due to dehydration, renal failure, high-protein diet, increased protein catabolism caused by tissue injury or massive bleeding into the alimentary tract. The reason of reduced urea level could be overhydration, low-protein diet or starvation and severe liver disease.

METHOD PRINCIPLE

Kinetic, enzymatic method with urease and glutamate dehydrogenase.



The rate of absorbance changing at 340 nm is proportional to the urea concentration.

Reagent Name	Pack Size
R1 Urea reagent	2 x 40 ml
R2 Urea reagent	2 x 10 ml
R3 Standard	2 ml

Please refer standard concentration mentioned in the vial.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate 1-UREA and 2-UREA reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-UREA with 1 part of 2-UREA. The working reagent should be prepared at least 30 min before use. Avoid foaming.

Stability of working reagent : 4 weeks at 2-8°C
5 days at 15-25°C

Protect from light and avoid contamination.

CONCENTRATIONS IN THE TEST

Tris (pH 7.6)	120 mmol/l
Glutamate Dehyrogenase (GLDH)	$\geq$ 1 KU/l
Urease	$\geq$ 20 KU/l
NADH	0.25 mmol/l
alpha-ketoglutarate	10 mmol/l

WARNINGS AND NOTES

- Product for in vitro diagnostic use only.
- The reagents are usable when the absorbance of the working reagent is higher than 1.200 (read against distilled water, wavelength λ=340 nm, cuvette l=1 cm, at temp. 25°C).
- The reagents contain 0.09% sodium azide as a preservative. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

-Automatic analyzer or photometer able to read at 340 nm
-Thermostat at 25°C or 30°C or 37°C,
-General laboratory equipment.

SPECIMEN

Serum, EDTA or heparinized plasma free from hemolysis, 24-hours urine. Do not use heparin ammonium salt and fluoride as anticoagulants.

Urine preparation: before analysis urine sample should be diluted 100-fold with 0.9% NaCl, and the results multiplied by 100. Mix well probes before measurement. 24-hours urine samples should be kept at 2-8°C preserved by maintenance of pH less than 4. Specimen can be stored up to 7 days at 2-8°C. Nevertheless it is recommended to perform the assay with freshly collected samples.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. **Programme Sheets** are available on request.

Wavelength	340 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvettes:

Reagent	Standard (S)	Test (T)
R1 Urea Reagent	800 µl	800 µl
R2 Urea Reagent	200 µl	200 µl
Bring to assay temperature, Then add		
R3 Standard	10 µl	-
Sample	-	10 µl

Mix well, after about 1 min (25-35°c) or 30-40 secs (37°C) read the absorbance A1 of the test (T) and standard (S) against air or water. After exactly 1 min. (for all temperature) read the absorbance A2 of the test (T) and standard (S). Calculate ΔA/min. (A1 - A2) for the test and standard.

CALCULATION

Urea concentration = ΔA (T) / ΔA(S) x standard concentration

1 mg of urea corresponds to 0.467 mg of urea nitrogen.

Serum	14 to 45 mg / dl
Urine	5 to 21 g/L

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

Sensitivity / Limit of Quantitation: 1.3 mg/dl.

Linearity: up to 250 mg/dl.

Specificity / Interferences

Haemoglobin up to 10 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 500 mg/dl do not interfere with the test.

The working reagent should not be used, if the absorbance is less than 1.000 against distilled water at 340 nm. It is strongly recommended that not to use haemolysed samples.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	Fixed Time (2-Point)
Wavelength	340 nm
Zero Setting	Distilled Water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	----
Delay Time	30 secs
Read Time	60 secs
No. of Reading	2
Interval Time	----
Sample Volume	0.01 ml (10 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer Standard vial
Units	mg/dl
Factor	----
Reaction Slope	Decreasing
Linearity	250 mg/dl

IVD

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