CATACHEM

BLOOD UREA NITROGEN (BUN)
MANUAL PROCEDURE
L-fork-A (Bulk)

Intended Use
For **IN VITRO quantitative** determination of Urea Nitrogen in serum.

Clinical Significance
Measurements of Urea Nitrogen activity are primarily used for diagnosing cardiac decompensation, increased protein catabolism, glomerulo-nephritis, chronic nephritis, polycystic kidney, nephrosclerosis, obstruction of the urinary tract, as well as for monitoring the causes and treatments. (1)

Method History
In 1942, Ormsby (2) applied the diacetyl monoxime reaction to the determination of urea. Colorimetric procedures have gradually been replaced by fully enzymatic procedures which offer milder reagents and improved specificity. Talke and Schubert's enzymatic procedure for urea nitrogen couples the urease hydrolysis of urea to the oxidation-reduction reaction of GLDH-NADH. (3) Catachem's Urea Nitrogen method for manual or automated applications is based upon the work reported by Talke and Schubert with adjustment for the use of Acetyl-NADH in a regenerative process.

Method Principle
The enzyme urease hydrolyzes urea to ammonia and carbon dioxide. The ammonia produced is quantitatively determined by the GLDH-Acetyl NADH reaction. The decrease in absorbance due to the oxidation of Acetyl-NADH to Acetyl-NAD is monitored at 340 or 380 nm. The reaction scheme illustrates the reactions that occur in this method:

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} 2NH_3 + C0_2$$

$$NH_3 + \alpha\text{-KG} + \text{Acetyl-NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + \text{Acetyl-NAD} + H_20$$

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients in the reagents will be approximately as follows.

Urea Nitrogen Reagent
Each liter contains:

Buffer	
Urease	$\geq$ 50,000 units
Alpha ketoglutarate	5.0 mmol/L
ADP	1.2 mmol/L
Acetyl-NADH	0.3 mmol/L
Glutamate Dehydrogenase	$\geq$ 12,000 units
Nonreactive ingredients and stabilizer	

Precautions
Avoid contact of reagents with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation of Working Reagent
Catachem BUN Reagent is packaged ready-for-use. No preparation is required.

Reagent Storage and Stability
Store the Catachem BUN reagent at 2-8°C. When stored as directed, this reagent is stable until the expiration date stated on the label. Upon opening and when stored as directed, the Working BUN Reagent is stable for 60 days at 2-8°C.

Specimen Collection and Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated, unhemolyzed serum, the Urea Nitrogen concentration is stable, when stored at 2-8˚C, for three days. (1)

Quality Control
To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances
Various substances have been reported to interfere with the Urea Nitrogen method. (1) A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (4)

Expected Values
The range of expected values determined for this method is 8-25 mg/dL for human samples. These values are suggested guidelines and vary with species. It is recommended that each laboratory establish the normal range for the species under test and for the area in which it is located.

Procedure
Important: Read entire instructions for procedure before proceeding with the assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.01 ml for sample
Cylinder	5 ml or 50 ml for reagent

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Materials Provided
Catachem Urea Nitrogen Reagent

Analytical Parameters

Wavelength	340nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	rate, first order, **inverse**
Reaction Time	120 seconds
Reagent Volume	1.0 ml
Sample Volume	0.01 ml
Total Volume	1.01 ml
Sample-to-reagent ratio	1:101

Assay Procedure
1. Set the spectrophotometer at 37˚C.
2. Pipette 1.0 ml of Urea Nitrogen Working Reagent into each of three cuvettes marked “Calibrator”, “Sample” and “Blank”.
3. Set spectrophotometer wavelength at 340nm and zero instrument with a cuvette containing water.
4. Pipette 0.01 ml of water to the cuvette marked “Blank”.
5. Pipette 0.01 ml of Calibrator or Sample into their respective cuvettes. Mix all cuvettes well.
6. At exactly 60 seconds, read and record the absorbance (A1).
7. At exactly 120 seconds after the A1 reading, read again and record the absorbance (A2).
8. Calculate the Urea Nitrogen concentration (mg/dL) in the sample(s) as shown in calculations and results.

Calculations and Results

$$\text{Urea (mg/dL)} = \frac{\text{Sample (A2 - A1)}}{\text{Calibrator (A2 - A1)}} \times \text{Calibrator (mg/dL)}$$

Example:

	A1	A2
Sample	0.01	0.03
Calibrator	0.01	0.05

Calibrator = 20 mg/dL

$$\text{Urea (mg/dL)} = \frac{0.03 - 0.01}{0.05 - 0.01} \times 20 \text{ mg/dL}$$

$$= 10 \text{ mg/dL}$$

Method Performance Characteristics
Sensitivity: 0.0016 - 0.0024 absorbance units/mg/dL
Linear Range: 0-200 mg/dL
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

BUN	Within-Run Precision		Total Precision	
Mean	**SD**	**CV**	**SD**	**CV**
mg/dL	**mg/dL**	**%**	**mg/dL**	**%**
6	**0.40**	**6.90**	**0.49**	**9.10**
58	**1.35**	**2.40**	**1.35**	**2.30**
117	**0.75**	**0.66**	**1.34**	**1.25**

Correlation
A comparison of this method using an automated analyzer was carried out against a reference method based on the procedure of Talke and Schubert resulted in the following regression statistics:

Range	=	4-150 mg/dL
N	=	100
Y	=	1.005 x - 0.38
r	=	0.994
Sy.x	=	3.9

References
1. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz, WB Saunders, Philadelphia (1976).
2. Ormsby A. A direct colorimetric method for the determination of urea in blood and urine. J Biol Chem 146:595-604 (1942).
3. Talke H, Schubert GE. Enzymatishche harnstoffbestimmung in blut and serum in optischen test NACH warburg. Klin Wchnsch 43, 174 (1965).
4. Young DS, Pestaner LC, Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 21(5): ID-432D (1975).

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