

Urea Nitrogen Reagent

(BUN)

Catalog #: 43724

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Urea Nitrogen in serum.

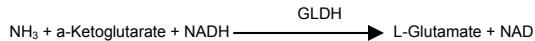
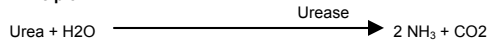
CLINICAL SIGNIFICANCE¹

Determination of urea nitrogen in serum is widely used as a screening test for renal function. When used in conjunction with the determination of creatinine in serum it is helpful in the differential diagnosis of the three types of azotemia; pre-renal, renal and post-renal.

METHODOLOGY

Urea has been determined by the direct method² where urea condenses with diacetyl to form a chromagen and an indirect method where ammonia is measured as a product of Urease action on urea.³ The liberated ammonia has been measured using Nessler's reagent⁴ and by the Berthelot reaction.⁵ Talke and Schubert introduced a totally enzymatic procedure in 1965 utilizing Urease and Glutamate Dehydrogenase.⁶ The present procedure is based on a modification of their method.

Principle



Urea is hydrolyzed by in the presence of water and 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 that is directly proportional to the urea nitrogen concentration in the sample.

REAGENT COMPOSITION

Active Ingredients:	Concentration
α -Ketoglutarate	7.5 mM
NADH	>0.20 mM
Urease (Jack Bean)	>8700 U/L
GLDH (Microbial)	>580 U/L
Non reactive stabilizers and fillers	
pH 8.35 \pm 0.1	

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion of reagent as toxicity has not yet been determined.
3. Reagents contain sodium azide (0.05%) as preservative. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.

REAGENT PREPARATION

Reagent comes in a ready to use form.

REAGENT STORAGE

Store the reagent at 2-8°C (refrigerated).
The reagent is stable until the expiration date stated on the label when stored at 2-8°C.

REAGENT DETERIORATION

The reagent should not be used if:
The reagent has a blank absorbance less than 1.4 at 340 nm.
The working reagent does not meet stated performance parameters.

SPECIMEN COLLECTION AND STORAGE⁷

1. Serum is recommended.

2. Plasma containing anticoagulants should not be used.
3. All material coming in contact with the sample must be free of ammonia and heavy metals.
4. Urea in serum is reported stable for seventy-two hours refrigerated at 2-8°C, and 6 months when frozen.
5. Specimen collection should be carried out in accordance with NCCLS M29-T2.⁸
6. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

INTERFERENCES:

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference (\pm 5%) from hemoglobin up to 600 mg/dL.

Bilirubin:

No significant interference (\pm 5%) from bilirubin up to 25.8 mg/dL.

Lipemia:

No significant interference from lipemia (\pm 5%) up to 1086 mg/dL measured as triglycerides.

For a comprehensive review of drug interference see Young, et al.⁹

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. An SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

Urea Nitrogen

TEMPERATURE:	37°C
WAVELENGTH:	340 nm
ASSAY TYPE:	Fixed Rate
DIRECTION:	Decrease
SAMPLE / RGT RATIO:	1 : 100
e.g. Sample Vol.	0.003 mL (3mL)
Reagent Vol.	0.300 mL (300 mL)
FIRST READ TIME:	30 Sec
DELAY TIME:	60 Sec
LAST READ TIME:	90 Sec

Procedure Note:

Calculations:

$$(\text{DA}/\text{min} = \text{A2} - \text{A1})$$

$$\frac{\text{DA}/\text{min} (\text{patient})}{\text{DA}/\text{min} (\text{standard})} \times \text{Concentration of standard} = \text{Urea Nitrogen (mg/dL)}$$

Example:
DA/min (patient) = 0.10
DA/min (standard) = 0.140
Concentration of standard = 40 mg/dL.

$$\frac{0.10}{0.14} \times 40 = 29 \text{ mg/dL Urea Nitrogen}$$

Limitations:

Samples with values exceeding 115 mg/dL should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

CALIBRATION

Use an aqueous Urea Nitrogen standard or an appropriate serum calibrator. Refer to appropriate instrument operator manual for recommended calibration interval.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Urea Nitrogen values.

EXPECTED VALUES

7-18 mg/dL⁷

It is strongly recommended that each laboratory establish its own reference range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 115 mg/dL.

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	42
Range of samples:	3 to 126 (mg/dL)
Correlation Coefficient:	0.9984
Slope:	0.9548
Intercept:	2.2 (mg/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (mg/dL)	6.1	12.2	51.9
S.D. (mg/dL)	0.2	0.5	0.4
C.V. (%)	3.7	4.1	0.9

Total

n=40 (10 days / 2 runs per day / 2 replicates per run)

Mean (mg/dL)	6.1	12.2	51.9
S.D. (mg/dL)	0.3	0.6	0.9
C.V. (%)	5.2	5.2	1.7

Sensitivity:

A calibration factor of approximately 796 was obtained, which is equivalent to a sensitivity of 1.26 D Abs per mg/dL.

REFERENCES

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Manufactured for:



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