SPECTRUM

The Creative Approach to Bioscience

Urea/BUN – Liquizyme (UV)

REF: 319 001	(3 x 50 ml)	150 test
REF: 319 002	(3 x 90 ml)	270 test
REF: 319 003	(4 x 100 ml)	400 test
REF: 319 004	(4 x 50 ml)	200 test
REF: 319 005	(8 x 50 ml)	400 test

Intended Use

Spectrum Diagnostics liquizyme urea reagent is intended for the in-vitro quantitative, diagnostic determination of urea in human serum or urine on both automated and manual applications.

Background

Urea is the major product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver and excreted through the kidneys. The circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur due to renal impairment or in some diseases such as diabetes, infection, congestive heart failure and during different liver diseases. Determination of blood urea nitrogen is the most widely used screening test for renal function together with serum creatinine.

Method

urease-UV fixed rate (enzymatic method).

Assay Principle

The series of reactions involved in the assay are as follows :

1. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide.

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

2. In the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH),the ammonia combines with α-ketoglutarate (α-KG) to produce L-glutamate.

$$2NH_4 + 2\alpha\text{-KG} + 2\ \text{NADH} \xrightarrow{\textit{GLDH}} 2\ \text{L-Glutamate} + 2\ NAD^+ + H_2O$$

The rate decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm.

Reagents

Standard urea (ST)

BUN	50	mg/dL
Urea	107	mg/dL

Reagent 1 (R1 Buffer)

Tris Buffer (pH 8.5)	50	mmol/L
α-Ketoglutarate	10	mmol/L
GLDH	8.0	K U/L
Urease	5.0	K U/L
Sodium azide	8.0	mmol/L

Reagent 2 (R2 Starter)

NADH	>0.20	mmol/L
Sodium azide	8	mmol/L

For further information, refer to the Urea/Bun reagent material safety data sheet.

Reagent Preparation

Prepare working solution as following:

REF: 319 001 : add 5 ml from R2 to one bottle of R1; mix gently
REF: 319 002 : add one bottle from R2 to one bottle of R1 ; mix gently
REF: 319 003 : add one bottle from R2 to one bottle of R1 ;mix gently
REF: 319 004 : add 5 ml from R2 to one bottle of R1; mix gently
REF: 319 005 : add 5 ml from R2 to one bottle of R1; mix gently

Or prepare the working solution according to the number of tests required by mixing 9 volumes of reagent 1 (R1) and 1volume of reagent 2 (R2) eg. 900 µl R1 +100 µl R2.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Both reagents (R1) and (R2) contain sodium azide which may react with copper or lead plumbing.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 – 8 °C.
Working solution is stable for 1 month at 2 – 8 °C or 8 days at 15 – 25 °C.
Once opened, the reagent vial and standard are stable for 3 months at the specified temperature if contamination is avoided.

Deterioration

Do not use liquizyme BUN reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

No special preparation of the patient is required. Use nonhemolyzed serum or plasma only.The only acceptable anticoagulants are heprin, EDTA and fluoride. Do not use ammonium heparin plasma.
Stability: 7 days at 15 –25 °C ; 7 days at 2 – 8 °C;
1 year at -20 °C
Urine samples are prediluted 1 : 50 with ammonium free water prior to assay.
Stability: 2 days at 15 – 25 °C ; 7 days at 2 – 8 °C;
1 month at -20 °C

System Parameters

Wavelength	340 nm
Optical path	1 cm
Assay type	Fixed Rate
Direction	Decrease
Sample : Reagent Ratio	1 : 100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
First read time	30 seconds
Delay time	60 seconds
Last read time	90 seconds
Temperature	37 °C
Zero adjustment	Against Air
Reagent Blank Limits	Low 1.00 AU
	High 2.0 AU
Sensitivity	0.9 mg/dL (0.15 mmol/L)
Linearity	200 mg/dL (33.2 mmol/L)

Procedure

	Standard	Specimen
Working solution	1 ml	1 ml
Standard	10 µl	------
Specimen	------	10 µl

Mix, and after 30 seconds read the absorbance A1 of the standard or specimen. Exactly 1 minute later, read the absorbance A2 of standard or specimen.

Calculation

Δ A specimen = A1 specimen – A2 specimen
Δ A standard = A1 standard – A2 standard

Serum urea concentration (mg/dl) = $\frac{\Delta A_{specimen}}{\Delta A_{standard}}$ x n

where n = 107 mg/dL

Urine urea concentration is determined by multiplying the result by the dilution factor (50).

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatiblity)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	45	150
SD	0.7	2.7
CV%	1.56	1.8

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	47	153
SD	0.82	2.81
CV%	1.74	1.84

Methods Comparison

A comparison between Spectrum Diagnostics Urea (UV) reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.992 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.9 mg/dL.

Linearity

The reaction is linear up to a urea concentration of 200 mg/dL. Specimens showing higher concentration should be diluted 1+2 with physiological saline and repeat the assay (result × 3).

Interfering Substances

Haemolysis
Erythrocyte contamination doesn't elevate results.Haemolytic specimens may cause high absorbance flagging.

Icterus
No significant interference.

Lipemia
Lipemic specimens may cause high absorbance flagging
Diluted sample treatment may be recommended.

Anticoagulants
Ammonium heparin should not be used.

Others
Ammonium ions should be avoided since it may cause erroneously elevated results.

Expected Values

Urea (Serum)
Adults <65 years : 15-50 mg/dL (2.5-8.33 mmol/L)
Adults >65 years : < 70 mg/dL (<11.66 mmol/L)

BUN (Serum)
Adults <65 years : 7-23.5 mg/dL
Adults >65 years : 7-32.9 mg/dL
Children : 5-18 mg/dL

Urine (24) hours
Urea : 20-35 g/24hrs (330-580 mmol/24hrs)
BUN : 9.3-16.4 g/24hrs

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure;interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

0.9 – 200 mg/dL (0.15 - 33.2 mmol/L).

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

Referances

1. Batton, C. J & Crouch, S.R: Anal. Chem. , 1977, 49:464-469 .
2. Shephard MD, Mezzachi RD: Clin Biochem Revs, 4:61-7, 1983.
3. Tiffany TO, jansen JM, Burtis CA,Overtion JB, SCOTT CD. Enzymatic kinetic rate and end point analyses of substrate, by use of a gemsaec fast analyzer. Clin Chem. 1972;18:829-840.
4. Tietz NW, Ed.Clinical guide to laboratory tests. 2ND. Philadelphia: WB Saunders;1990:566.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
319 001	3 x 50 ml
319 002	3 x 90 ml
319 003	4 x 100 ml
319 004	4 x 50 ml
319 005	8 x 50 ml

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