SPECTRUM

The Creative Approach to Bioscience

Urea/BUN - Liquizyme (Modified Urease-Berthlot Method)

REF: 318 001	100 test	REF: 318 002	200 test
R1 Buffer	1 x 100 ml	R1 Buffer	2 x 100 ml
R2 Urease	1 x 6 ml	R2 Urease	2 x 6 ml
R3 Alkaline reagent	1 x 20 ml	R3 Alkaline reagent	1 x 45 ml
REF: 318 003	500 test	REF: 318 004	1000 test
R1 Buffer	5 x 100 ml	R1 Buffer	4 x 250 ml
R2 Urease	2 x 15 ml	R2 Urease	51 ml
R3 Alkaline reagent	2 x 55 ml	R3 Alkaline reagent	1 x 210 ml

Intended Use

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

Background

Urea is the major end 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-colorimetric method.

Assay Principle

The reaction involved in the assay system is as follows:
Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide.

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

The free ammonia in an alkaline pH and in the presence of indicator forms coloured complex proportional to the urea concentration in the specimen.

Reagents

Standard urea (ST) Aqueous primary standard

50 mg/dL	8.33 mmol/l

Reagent 1 (R1 Buffer)

Phosphate buffer pH 8.0	100 mmol/l
Sodium salicylate	80 mmol/l
Sodium nitroprusside	6.0 mmol/l
EDTA	30.0 mmol/l

Reagent 2 (R2 Enzyme)

Urease	>6000 U/l

Reagent 3 (R3 Alkaline Reagent)

Sodium hydroxide	400 mmol/l
Sodium hypochlorite	20.0 mmol/l

Irritant (xi) R36/38: Irritating to eyes and skin. **S26:** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. **S37/39:** Wear suitable gloves and eye/face protection.

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

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.

Reagent Preparation, Storage and Stability

Spectrum colorimetric urea reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles (2 – 8 °C).
Once opened, the reagent vial and standard are stable for 3 months at the specified temperature if contamination is avoided.
NB: For mega labs having high numbers of patient specimens,working buffer reagent can be prepared .(**Stability 1 week**)

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		

REF:318 001: add 5 ml from R2 to one bottle of R1; mix gently.
REF:318 002: add 5 ml from R2 to one bottle of R1; mix gently.
REF:318 003: add 5 ml from R2 to one bottle of R1; mix gently.
REF:318 004: add 12.5 ml from R2 to one bottle of R1; mix gently.

Deterioration

Do not use the reagent if it is turbid. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Serum
No special preparation of the patient is required. Use non haemolyzed serum only. 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
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	578 nm (578-623 nm)
Optical path	1 cm
Assay type	End-point
Direction	increase
temperature	15-25 °C or 37 °C
Zero adjustment	Against Reagent blank
Reagent Blank Limits	Low 0.02 AU High 0.2 AU
Sensitivity	0.6 mg/dL (0.1 mmol/l)
Linearity	200 mg/dL (33.3 mmol/l)

Procedure 1

	Blank	Standard	Specimen
R1(Buffer)	1.0 ml	1.0 ml	1.0 ml
R2(Enzyme)	one drop (50 µl)	one drop (50 µl)	one drop (50 µl)
Standard	-----	10 µl	-----
Sample	-----	-----	10 µl
Mix and incubate for at least 3 minutes at 37 °C or 5 minutes at 20-25 °C.			
R3(Alk.Reagent)	200 µl	200 µl	200 µl
Mix and incubate for 5 minutes at 37 °C or 10 minutes at 20-25 °C Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.			

Procedure 2 (Using working solution)

	Blank	Standard	Specimen
Working solution	1.0 ml	1.0 ml	1.0 ml
Standard	-----	10 µl	-----
Sample	-----	-----	10 µl
Mix and incubate for at least 3 minutes at 37 °C or 5 minutes at 20 -25 °C.			
R3(Alk.Reagent)	200 µl	200 µl	200 µl
Mix and incubate for 5 minutes at 37 °C or 10 minutes at 20-25 °C. Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.			

Calculation

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

where n = 50.0 mg/dl (8.33 mmol/l)

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

Urea Nitrogen: To convert the result from urea to urea nitrogen multiply the result by 0.467.

Quality Control

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

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	60	144
SD	1.87	2.1
CV%	3.12	1.46

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	62	146
SD	1.92	2.5
CV%	3.25	1.65

Methods Comparison

A comparison between Spectrum Diagnostics Urea/BUN reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.97 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.6 mg/dL.

Linearity

The reaction is linear up to a urea concentration of (200 mg/dl) 33.3 mmol/L. Specimens showing higher concentrations should be diluted 1+2 with physiological saline and repeat the assay (result×3).

Interfering Substances

Haemolysis
Erythrocyte contamination doesn't elevate results.

Icterus
No significant interference.

Lipemia
Lipemic specimens interfere with the method of Berthlot.

Anticoagulants
Ammonium heparin should not be used.

Others
Ammonium ions should be avoided since it may cause erroneously elevated results. Color development in the Berthlot reaction is suppressed by amines, thiols, steroids and ascorbic acid.

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

0.6 – 200 mg/dL (0.1 - 33.3 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.

References

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. Tietz NW, ED. Clinical guide to Laboratory tests. 2ND ED. Philadelphia: WB Saunders; 1990:566.
4. Tiffany to, jansen JM, Burtis CA,Overton JB, Scott CD. Enzymatic Kinetic Rate and end Point analyses of Substrate, By USE of A Gemsaec fast analyzer. Clin Chem.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
318 001	100 Test
318 002	200 Test
318 003	500 Test
318 004	1000 Test

Spectrum For Diagnostic Industries - Free Zone
Ismailia Free Zone Industrial Area, Block 5 .
Cairo- Port said Avenue.
Ismailia,Egypt
Tel: +2 064 3488 013 - +2 064 3488 014 Fax: +2 064 3488 015
www.sdi-fz.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

CE

ISO 13485:2016