Liquid Urea Nitrogen (BUN) Reagent Set

Intended Use
For the quantitative determination of urea nitrogen in serum using the Mindray BS-480 analyzer. For *in vitro* diagnostic use only.

Clinical Significance
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.[1]

Method History
Urea has been determined by the direct method[2] 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 reagent[4] 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
$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \alpha\text{-Ketoglutarate} + NADH + H^+ \xrightarrow{\text{GD}} \text{L-glutamate} + NAD^+ + H_20$$

Urea is hydrolyzed by 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
Working reagent concentrations: Urease (Jack Bean) >15,000 U/L, GLDH (Bovine) >200 U/L, ADP >0.6 mM, α-Ketoglutarate 3.4 mM, NADH >0.28 mM, Buffer, pH 7.8 ± 0.1, stabilizers, Sodium Azide (0.28%) as preservative.

Reagent Preparation
The reagents are ready to use.

Reagent Storage
Store R1 and R2 reagents at 2-8°C. The reagents are stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Reagent Deterioration
The reagent should not be used if the working reagent has a reagent blank absorbance less than 1.0 at 340 nm.

Precautions and Hazards
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 (0.28%) as preservative. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.
4. All specimens should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

Hazards:

R1: Hazard Classifications: Specific Target Organ Toxicity, Single Exposure; Respiratory System (Category 3)
Hazard Statement: H335: May cause respiratory irritation.
Precautionary Statements: Prevention: P261 Avoid breathing dust/fume/gas/mist/vapors/spray. P271 Use only in a well-ventilated area. Response: P312 Call a POISON CENTER or doctor/physician if you feel unwell. P304 + P340 IF INHALED: Remove victim to fresh air and Keep at rest in a position comfortable for breathing. Storage: P403 + P233 Store in a well-ventilated place. Keep container tightly closed. Disposal: P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations.

Signal Word: Warning

R2: Hazard Classifications: Acute Toxicity, Dermal (Category 4)
Hazard Statement: H312: Harmful in contact with skin.
Precautionary Statements: Prevention: P280 Wear protective gloves/protective clothing/eye protection/face protection. Response: P312 Call a POISON CENTER or doctor/physician if you feel unwell. P363 Wash contaminated clothing before reuse. P302 + P352 IF ON SKIN: wash with plenty of soap and water. Disposal: P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations.

Signal Word: Warning

Specimen Collection and Storage
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.[7]
4. Urea in serum is reported stable for seventy-two hours refrigerated at 2-8°C. Unrefrigerated sera should be used within eight hours.
5. Specimen collection should be carried out in accordance with NCCLS M29-T2.[8] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences

1. Urease action is inhibited by fluoride.
2. Samples with abnormal ammonia levels give falsely elevated BUN results.
3. Bilirubin to the level of 20 mg/dl was found to exhibit negligible interference (<2%) in this assay.
4. Hemoglobin to the level of 200 mg/dl was found to exhibit negligible interference (<5%) in this assay.
   NOTE: The BUN level was 46.0 mg/dl for the Bilirubin study and 46.3 mg/dl for the Hemoglobin study.
5. For a comprehensive review of drug interference see Young, et al.[9]

Materials Provided

Urea Nitrogen Enzyme Reagent (R1), Urea Nitrogen Coenzyme Reagent (R2)

Materials Required but not Provided

1. Mindray BS-480 Analyzer
2. BS-480 Operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry control, catalog number CHEQ480

Limitations

Samples with values above 150 mg/dl should be diluted with 0.9% saline 1:1, re-assayed and the results multiplied by two.

Calibration

Use MedTest DX Chemistry Calibrator (Catalog Number: CHEC480). The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Quality Control

The validity of the reaction should be monitored by use of the control sera with known normal and abnormal BUN values. These controls should be run at least with every working shift in which urea nitrogen assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values

7-18 mg/dl[7]
It is strongly recommended that each laboratory establish its own reference range.

Performance

1. Assay Range: 1-150 mg/dl. Samples that exceed 150 mg/dl should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: A study was performed between the Mindray BS-480 and a similar analyzer using this method resulting in the following:

Method	BUN
N	84
Mean BUN (mg/dL)	27.7
Range (mg/dL)	5-149
Standard Deviation	29.0
Regression Analysis	y = 0.994x – 0.7
Correlation Coefficient	0.9962

3. Precision: Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	12.7	47.4	131.9
Standard Deviation	0.7	0.7	0.9
Coefficient of Variation (%)	5.2%	1.4%	0.6%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	13.0	48.8	134.3
Standard Deviation	0.8	1.7	2.5
Coefficient of Variation (%)	7.2%	2.5%	1.9%

4. Sensitivity: 2SD limit of detection (95% Conf) = 1 mg/dL

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia W.B. Saunders (1976).
2. Fearon, W.R., Biochem J. 331:902 (1939).
3. Marshall, E.K., Jr., J. Biol. Chem. 15:487 (1913).
4. Gentzkow, C.J., J. Biol. Chem. 143:531 (1952).
5. Fawcett, J.K., Scott, J.E., J. Clin. Path. 13:156 (1960).
6. Talke, H., Schubert, G.E., Klin. Wschr. 43:174 (1965).
7. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia W.B. Saunders, p991 (1976).
8. NCCLS document "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
9. Young, D.S., et al, Clin. Chem. 21:1D (1975).
10. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

MEDTEST DX
A MEDTEST COMPANY

Liquid Urea Nitrogen (BUN) Reagent Set

CHEMISTRY PARAMETERS

Chem:	BUN	No.:	206	Sample Type:	Serum
Chemistry:	Urea Nitrogen (Liquid)			Print Name:	BUN
Reaction Type:	Fixed Time			Reaction Direction:	Negative
Pri Wave:	340			Sec Wave:	660
Unit:	mg/dL			Decimal	0
Blank Time:	47 49			Reaction Time:	55 63

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent
Standard:	1.5 ul	--- ul	--- ul	R1:	150 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2:	38 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3:	--- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4:	--- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	1	150	Linearity Limit:	
Linearity Range (Decreased)	____	____	Substrate Depletion:	
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

5449 Research Dr. • Canton MI 48188 • USA • Phone: 734-487-8300 • Toll Free: 800-445-9853 • Fax: 734-483-1592 • www.medtestdx.com

Liquid Urea Nitrogen (BUN) Reagent Set

CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	Pos
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	BUN	0	mg/dL
Chemistry Calibrator	*	*	*	BUN	*	mg/dL

Calibration Setup

Chem: BUN

Calibration Settings

Math Model:	Two-Point Linear		
Factor:		Replicates:	2

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.

* Indicates user defined parameter.

REF BUN480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	