Pointe Urea Nitrogen (BUN) Reagent Set

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Intended Use

For the quantitative determination of urea nitrogen in serum using the Yumizen C230 and Yumizen C240 analyzers. For *in vitro* diagnostic use only. **Rx 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, 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.

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

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.

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. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

Test Parameters

Test:	Urea Nitro	Chemistry:	Urea Nitrogen
Chemistry No.:	206	Print Name:	Urea Nitrogen
Reaction Type:	Fixed-Time	Reaction Direction:	Negative
Pri. Wave:	340 nm	Sec. Wave:	670 nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	2 7
Unit:	mg/dL	Incubation Time:	3

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	2	uL	ulL	uL	R1: 200	uL	uL
Decreased;		uL	uL	uL	R2: 50		
Increased;		uL	uL	uL			

Linearity Range (Standard);		0-150	Linearity Limit:			
Linearity Range (Decreased):			Substrate Depletion:			
Linearity Range (Increased):			Mixed Blank Abs.:	- 40000	40000	
R1 Blank Abs.:	- 40000	40000	On-board Stability:	30	Day (s)	
Blank Response	- 40000	40000	Reagent Alarm Limit:	5		
Twin Chemistry:						

Prozone Check:			
Q1:	Q2:	Q3:	
Q4:	PC:	ABS:	

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Uint
	1	0	mg/dL

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

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Calibration Setup Parameters

Chem: BUN
Calibration Setting
Math Model: Two Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability:
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

* User Defined

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 an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Calculation (Example)
(A_1-A_2) = Absorbance change between readings

$$\frac{(A_1 - A_2)\ \text{unknown}}{(A_1 - A_2)\ \text{standard of standard}} \times \text{concentration} = \text{BUN (mg/dl)}$$

Example: If the unknown had an $A_1 = 1.5$ and $A_2 = 1.0$,
the standard $A_1 = 1.5$ and $A_2 = 0.9$ and
the concentration of the standard = 20 mg/dl then:

$$\frac{(1.5 - 1.0)}{(1.5 - 0.9)} = \frac{0.5}{0.6} \times 20 = 17 \text{ mg/dl}$$

NOTE: To obtain results in SI units multiply by 10 to convert dl to liters and divide by 28, the molecular weight of nitrogen.

Example: 17 mg/dl x 10/28 = 6.06 mmol/L.

To convert mg/dl Urea Nitrogen to mmol Urea/L, multiply the mg/dl Urea Nitrogen value by 0.357.

To convert mg/dl Urea Nitrogen to mg/dl Urea, multiply the mg/dl Urea Nitrogen value by 2.14.

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: 0-150 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 Yumizen 200 series and a similar analyzer using this method, resulting in a correlation coefficient of 0.986 and the regression equation of y = 0.95x + 0.6.
3. Precision: Precision studies were performed using the Yumizen 200 series analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
15.6	0.5	3.2	14.1	0.8	5.7
55.3	1.2	2.1	51.1	1.8	3.5

4. Sensitivity: The sensitivity for the Liquid BUN reagent was investigated by reading the change in absorbance at 340 nm for a saline sample, and serum samples with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Liquid BUN reagent showed little or no drift on a zero sample. Under the reaction conditions described, 1mg/dl of BUN gives an absorbance of 0.003.

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).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-B7552-150

Manufactured by
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IVD

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CE

Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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