

Intended Use

For the quantitative determination of urea nitrogen in serum using the Yumizen C560 analyzer. **Rx Only.**

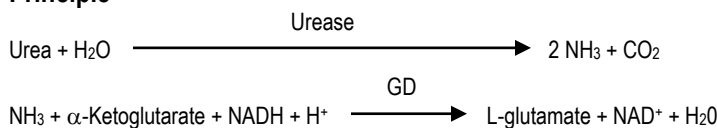
Clinical Significance

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 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 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 \pm 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.

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. **Refer to the Safety Data Sheet for this product (SDS-B7552) available by calling 1-734-487-8300.**



Signal Word: Warning



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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.

Pointe Urea Nitrogen (BUN) Reagent Set

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 C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

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 Pointe Chemistry Calibrator (Catalog Number: C7506-50). 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/dl⁷

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 Yumizen C560 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 Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.¹⁰

Sample	Within Day		
	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%

Sample	Total		
	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).

CHEMISTRY PARAMETERS

Chem:	Urea Nitro	No.:	206	Sample Type:	Serum
Chemistry:	Urea Nitrogen (Liquid)			Print Name:	Urea Nitro
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: --- ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	<input type="checkbox"/> Sample Blank	<input checked="" type="checkbox"/> Auto Rerun		R4: --- ul	--- ul
 <u>Slope/Offset Adjustment</u>					
	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:			<input type="checkbox"/> Enzyme Linear Extension
<input type="checkbox"/> Prozone Check		<input type="radio"/> Rate Check	<input type="radio"/> Antigen Addition
Q1:	Q2:	Q3:	Q4:
PC:	ABS:		

Pointe 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						
<u>Calibrator</u>	<u>Pos</u>	<u>Lot No</u>	<u>Exp Date</u>	<u>Chem</u>	<u>Conc</u>	<u>Unit</u>
Water	W	*	*	BUN	0	mg/dL
Chemistry Calibrator	*	*	*	BUN	*	mg/dL
Calibration Setup						
Chem:	Urea Nitro					
Calibration Settings						
Math Model:	Two-Point Linear					
Factor:		Replicates:	2			
Acceptance Limits						
Cal Time:	336	Hour				
Slope Diff:	---	SD:	---			
Sensitivity :	---	Repeatability:	---			
Deter Coeff:	---					
Auto Calib.						
<input type="checkbox"/> Bottle Changed	<input type="checkbox"/> Lot Changed	<input type="checkbox"/> Cal Time				

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-B7552-600  Manufactured by HORIBA Instruments Inc.- Pointe Brand
5449 Research Drive Canton, MI 48188

  **IVD**

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.

Manufactured by HORIBA Instruments Incorporated – Pointe Brand
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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 **Rx Only:** Prescription Use Only