

Intended Use

For the quantitative determination of urea nitrogen in serum. For *in vitro* diagnostic use only.

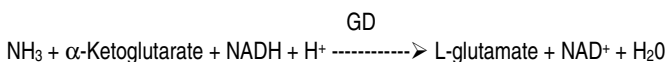
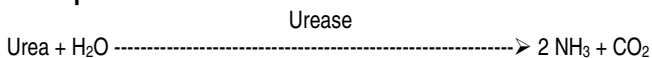
Clinical Significance

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 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) 1667 U/L, ADP 2.0 mM, α -Ketoglutarate 4.0 mM, NADH 0.28 mM, Buffer, pH 7.8 \pm 0.1, stabilizers, Sodium Azide (0.2%) as preservative.

Reagent Preparation

Prepare working reagent by mixing 5 parts Enzyme reagent (R1) with 1 part Coenzyme (R2) reagent.

Reagent Storage

1. Store R1 and R2 reagents at 2-8°C.
2. Store working reagent at 2-8°C.
3. Working reagent is stable 3 days at 18-25°C and 14 days at 2-8°C.

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 (0.2%) 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. Accurate pipetting devices. (10ul and 1.0ml)
2. Timer. (Able to measure 30 and 60 second intervals)
3. Test tubes/rack
4. Spectrophotometer with a temperature controlled cuvette able to measure at 340nm

Procedure (Automated-General)

Wavelength:	340 nm
Assay Type:	Initial Rate
Sample/Reagent Ratio:	1:101
Reaction Direction:	Decreasing
Temperature:	37°C
Lag Phase:	30 seconds
Read Time:	60 seconds
Low Normal:	7 mg/dl
High Normal:	18 mg/dl

Application Parameters for various automated instruments are available. Please contact the manufacturer's Technical Service Department for specific information.

Liquid Urea Nitrogen (BUN) Reagent Set

Procedure (Manual)

1. Prepare working reagent according to instructions.
2. Zero spectrophotometer with water at 340 nm.
3. Pipette 1.0ml of reagent into test tubes and allow reagent to come to 37°C.
4. Add 0.01ml (10ul) of calibrator, control, or sample to test tube and immediately place in the spectrophotometer.
5. After thirty seconds read and record the absorbance (A_1).
6. Sixty seconds after the first reading take another reading (A_2).
7. Determine the absorbance change between the two readings (A_1-A_2).
8. Repeat procedure for each sample.
9. See "Calculations" for determination of results.

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 BUN standard (20 mg/dl) or 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

(A_1-A_2) = Absorbance change between readings

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

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: $17 \text{ mg/dl} \times 10/28 = 6.06 \text{ mmol/L}$.

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 their own frequency of control determination.

Expected Values

7-18 mg/dl⁷

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: Results obtained with this reagent (y) in 107 samples ranging in BUN levels from 5 to 139 mg/dl were compared with those obtained in the same samples using a dry powder reagent (x) from another manufacturer, based on the same methodology. The correlation coefficient was 0.999 and the regression equation of $y = 1.05x - 0.2$. ($S_y - x = 20.93$).
3. Precision: Precision studies were performed following a modification of the guidelines which are contained in the NCCLS document EP5-T2¹⁰

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
10.1	0.3	3.1	10.3	0.5	4.6
68.4	0.9	1.4	69.3	0.9	1.3
144.3	1.9	1.3	145.1	2.3	1.6

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, 1 mg/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. Gentskow, 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).

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