STANBIO LABORATORY
An EKF Diagnostics Company

CE

Stanbio Urea Nitrogen (BUN) Liqui-UV®
Procedure No. 2020

For the Kinetic Quantitative Determination of Urea Nitrogen (BUN) in Serum

Summary and Principle

Urea is the principle waste product of protein catabolism. It is synthesized in the liver from ammonia which is produced as a result of the deamination of amino acids. Normally, urea nitrogen in the blood comprises only about 45% of the non-protein nitrogen. The importance of urea nitrogen determination is its value as an indicator of liver and kidney functions. Decreases in Blood Urea Nitrogen (BUN) are seen with nephritis, acute liver destruction, amyloidosis and pregnancy.[1] Increases in BUN are encountered with acute and chronic nephritis, intestinal and urinary obstruction, uremia, metallic poisoning, pneumonia, Addison's Disease, peritonitis, surgical shock and cardiac failure.

The Stanbio BUN procedure is a modification of the method described by Sampson.[1] Urea is catalytically converted to ammonium carbonate by the use of urease. The reaction rate is dependent upon the concentration of the influence of glutamic dehydrogenase. The rate of this second reaction is dependent upon the first and can be measured by the rate of conversion of NADH to NAD by the change of absorbancy at 340 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + 2HO_3$$

$$2\text{-Oxoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + \text{NAD}^+ + H_2O$$

Reagent

BUN Buffer (R1), Ref. No. 2021

Composition:		
Tris, pH 7.8	120	mmol/L
2-Oxoglutarate	7.0	mmol/L
ADP (monosodium salt)	0.6	mmol/L
Urease (Jack Bean)	60000	U/L
GLDH (Baker's yeast)	10000	U/L

BUN Enzyme (R2), Ref. No. 2022

Composition:		
NADH (Disodium salt)	0.25	mmol/L

BUN Standard - 30 mg/dL, Ref. No. 1022

Aqueous solution of urea in addition to trace amounts of stabilizers. Standard is referenced against NIST material.

Precautions: The reagents are for "In Vitro Diagnostic Use". Normal precautions exercised in handling laboratory reagents should be followed. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Reagent Preparation: Buffer and Enzyme liquid reagents are supplied ready-to-use. Prepare Working Reagent in the ratio of 5 parts Buffer (R1) to 1 part Enzyme (R2), (i.e., 25 mL Buffer and 5 mL Enzyme). Before use allow to stand for at least 30 minutes at room temperature (15-25°C).

Reagent Storage and Stability: Reagents are stable until the expiration date on their respective labels, when properly stored at 2-8°C and protected from light. Reagents should appear clear and colorless. Discard if either appears cloudy or contains particulate matter. The Working Reagent is stable for 4 weeks at 2-8°C or 5 days at room temperature (15-25°C). Protect the reagent from direct light. The Working Reagent should be discarded if the initial absorbance, read against distilled water at 340 nm, is below 1.000.

Material Required But Not Provided

Spectrophotometer capable of absorbance reading at 340 nm
Constant temperature block or bath, 37°C, or temperature controlled cuvet
Accurate pipetting devices Test tubes Interval timer

Specimen Collection and Storage

Non-hemolyzed serum is the specimen of choice. Whenever possible specimens should be separated and analyzed on the day of collection. Anticoagulants containing ammonium or fluoride salts must be avoided.[2] Urea in serum is stable for up to 24 hours at room temperature (15-25°C), several days refrigerated at 2-8°C and for at least 2-3 months when frozen (-20°C). Bilirubin levels up to 40 mg/dL, hemoglobin levels up to 200mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test.[4]

Interfering Substances: In addition to hemolysis, fluoride at elevated concentrations and ammonia can cause interferences.[3]

Manual Procedure

1. Prepare BUN Working Reagent according to instructions.
2. Zero spectrophotometer at 340 nm with distilled water.
3. For each sample and control, add 1.0 mL Working Reagent to cuvet or test tube and warm to 37°C for 3 minutes.
4. Add 10 µL (0.010 mL) serum to its respective tube and mix gently.
5. After exactly 30 seconds read and record absorbance (A_1).
6. At exactly 60 seconds after reading (A_1), read and record absorbance (A_2).
7. Calculate change in absorbance (ΔA) by subtracting (A_1-A_2).
8. Run patients and controls by following steps 4 through 7.

NOTE: If cuvet is not temperature controlled, incubate samples at 37°C between readings.

Quality Control: Stanbio Ser-T-Fy® I, Normal Control Serum, Cat. No. G427-86 and Ser-T-Fy® II, Abnormal Control Serum, Cat. No. G428-86 are recommended for each run. Other commercially available controls with BUN values assayed by this method are also suitable. BUN activity determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

Calibration: Calibration is required. The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer.

Results

Values are derived by comparing the absorbance change(ΔA) of the unknown (u) with that of a standard (s) identically treated.

$$\text{Serum BUN (mg/dL)} = \frac{\Delta Au}{\Delta As} \times 30$$

Where Au and As are the absorbance changes (decrease) of unknown and standard, respectively, and 30 the concentration of standard (mg/dL)

Example: $\Delta Au = 0.045$, $\Delta As = 0.090$

$$\text{Serum BUN (mg/dL)} = \frac{0.045}{0.090} \times 30 = 15$$

Limitations

If the BUN value exceeds 140 mg/dL the specimen should be diluted 2-fold (1+1) with distilled water, the assay repeated and results multiplied by the dilution factor of 2. BUN values for neonatal patients have not been established with this procedure.

Expected Values[3]

Normal Range:

BUN	8 - 23 mg/dL
Urea	17 - 49 mg/dL

Urea (mg/dL) = BUN (mg/dL) x 2.14
Urea (mmol/L) = Urea(mg/dL) x 0.167

This range should serve only as a guideline. It is ultimately the responsibility of the laboratory to establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics[4]

The following performance characteristics were performed using an Epos 5060

Comparison: A group of 68 sera ranging in BUN values from 11 - 97 mg/dL was assayed by the described BUN method and by a similar commercially available BUN reagent. Comparison of the results yielded a correlation coefficient of 0.999 and the regression equation was y = 1.012x - 0.47. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

Precision: Within-run precision was established by 20 assays on three different levels of commercial serum controls. Total Precision values were obtained by assaying 3 commercial controls for 5 consecutive days.

Within-Run

	Serum 1	Serum 2	Serum 3
Mean BUN (mg/dL)	22	45	54
Std. Deviation (mg/dL)	0.6	0.8	0.6
C.V. (%)	2.6	1.8	1.2

Total Precision

	Serum 1	Serum 2	Serum 3
Mean BUN (mg/dL)	15	46	70
Std. Deviation (mg/dL)	0.4	0.6	0.7
C.V. (%)	3.0	1.2	1.0

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.
Linearity: Linear from 2-140 mg/dL. Performed according to NCCLS Guidelines EP6-P.
Sensitivity: Based on an instrument resolution of A = 0.001, the method presented shows a sensitivity of 2.0 mg/dL. Values less than 2.0 mg/dL should be reported as < 2.0 mg/dL.

References

1. Sampson, E.J., Baird, M.A., Burtis C.A., et al.: A coupled-enzyme equilibrium method for measuring urea in serum: Optimization and evaluation of the AACC Study Group on urea candidate reference method. Clin Chem., 26, 816-826, 1980.
2. Tietz, N.W., Textbook of Clinical Chemistry, W.B. Saunders Company, Philadelphia, PA. (1986), p.1270-1271.2.
3. Henry, R.J., Clinical Chemistry Principles and Techniques, 2nd Edition, Harper and Row, Hagerstown, NY, (1974), p.511.
4. Stanbio Laboratory Data

Manufactured By:
Stanbio Laboratory • 1261 North Main Street • Boerne, Texas 78006 USA
Ph: (830) 249-0772 • Fax (830) 249-0851 • e-mail: stanbio@stanbio.com
http://www.stanbio.com
DN: RBR.2020CE.02 • Last Revision: 04/04 • Procedure No. 2020CE

STANBIO LABORATORY
An EKF Diagnostics Company