INTENDED USE
Urea nitrogen (BUN) liquid reagent is used for the kinetic quantitative determination of Urea Nitrogen (BUN) in human serum used in routine examination and monitoring of therapy and relapses.

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. 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.

This 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 absorbency at 340 nm.

1. Urea + H2O ——> 2NH3+ + CO2
2. 2-Oxoglutarate + NH3+ + NADH + H+ ——> GLDH L-Glutamate + NAD+ + H2O

REAGENTS
BUN Liquid Reagents 1 and 2 and BUN Standard come in separate containers, and all reagents are clear, colorless liquid in ready to use format.
1. After combining BUN Liquid R1 and BUN Liquid R2 as directed the reagent contains:
   - TRIS Buffer, pH 7.8 100 mmol/L
   - 2-Oxoglutarate 5 mmol/L
   - ADP 0.6 mmol/L
   - Urease >20,000 U/L
   - GLDH >1,500 U/L
   - NADH 0.25 mmol/L
   - Stabilizers, Preservatives
2. Urea Nitrogen Standard (20 mg/dL)

WARNINGS AND PRECAUTIONS
1. The reagents are for in vitro diagnostic use and are intended for professional use only.
2. Normal precautions exercised in handling laboratory reagents should be followed.
3. 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.
4. Refer to Material Safety Data Sheet for any updated risk, hazard, or safety information.

REAGENT PREPARATION
1. The working reagent is prepared by mixing five (5) volumes of R1 with one (1) volume of R2 in a disposable container. Example: 25 ml R1 + 5 ml R2.
2. Urea Nitrogen Standard is ready to use.

REAGENT STORAGE AND STABILITY
Both the BUN reagents and standard 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 2 weeks at 2-8°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.

MATERIALS REQUIRED BUT NOT PROVIDED
- Spectrophotometer capable of absorbance reading at 340 nm
- Constant temperature block or bath, 37°C, or temperature controlled cuvette
- 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.3 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).

INTERFERING SUBSTANCES
Fluoride and ammonia cause interference with the BUN assay. Blood collected in tubes containing ammonium heparinate should NOT be used. Young, et al4 provide a list of drugs and other substances that interfere with the determination of BUN.

MANUAL PROCEDURE
1. Prepare BUN working reagent according to instructions.
2. Pipette 1.0 mL of working reagent into tubes labeled “standard”, “control”, “patient”, etc.
3. Pre-incubate all tubes at 37°C for at least five minutes.
4. Zero spectrophotometer at 340 nm with distilled water.
5. Add 10 µL (0.010 mL) of sample, mix and return to a thermo cuvette.
6. After exactly 30 seconds, read and record absorbance (A1).
7. At exactly 60 seconds after reading (A1), read and record absorbance (A2).
8. Repeat steps 5-7 for all test specimens and standard*.
9. Calculate change in absorbance (ΔA= A2 - A1) per minute.
10. See “Calculations”.

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

* TC MULTI-PURPOSE CALIBRATOR MAY BE USED IN PLACE OF STANDARD.
AUTOMATED PROCEDURE
Refer to appropriate application manual available.

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established BUN values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

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

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

Serum BUN (mg/dL) = \( \frac{\Delta A_u \text{ Unknown} \times \text{Conc. Std.}}{\Delta A_s \text{ Std. or Cal.}} \)

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

Example: \( \Delta A_u = 0.035 \), \( \Delta A_s = 0.045 \), Conc. Std. = 20 mg/dL

Serum BUN (mg/dL) = \( \frac{0.035 \times 20}{0.045} = 15.6 \text{mg/dL} \)

NOTE: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.357

LIMITATIONS
If the BUN value exceeds 80 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 VALUES²
Normal Range: BUN 8 - 23 mg/dL
Urea 17 - 49 mg/dL
Urea (mg/dL) = BUN (mg/dL) \times 2.14
Urea (mmol/L) = Urea(mg/dL) \times 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
1. Comparison: A group of 40 sera ranging in BUN values from 5.9-108.5 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 0.994 and the regression equation was \( y = 0.962x - 0.721 \). (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

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

Within-Run

<table>
<thead>
<tr>
<th></th>
<th>Serum 1</th>
<th>Serum 2</th>
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</thead>
<tbody>
<tr>
<td>Mean (mg/dL)</td>
<td>12.9</td>
<td>51.8</td>
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<tr>
<td>Std. Deviation (mg/dL)</td>
<td>0.33</td>
<td>0.74</td>
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<tr>
<td>C.V. (%)</td>
<td>2.62</td>
<td>1.43</td>
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Run-to-Run

<table>
<thead>
<tr>
<th></th>
<th>Serum 1</th>
<th>Serum 2</th>
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<tbody>
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<td>Mean (mg/dL)</td>
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<td>44.6</td>
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<tr>
<td>Std. Deviation (mg/dL)</td>
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<td>0.75</td>
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<tr>
<td>C.V. (%)</td>
<td>1.20</td>
<td>1.67</td>
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</table>

Precision studies were performed according to NCCLS Tentative Guideline EP5-T.

REFERENCES