Urea/BUN - LS
(Modified Urease-Berthlot Method)

**Intended Use**
Spectrum Diagnostics colorimetric urea reagent is intended for the in-vitro quantitative, diagnostic determination of urea in human serum on both automated and manual systems.

**Background**
Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver and excreted through the kidneys. The circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur due to renal impairment or in some diseases such as diabetes, infection, congestive heart failure and during different liver diseases. Determination of blood urea nitrogen is the most widely used screening test for renal function together with serum creatinine.

**Method**
Urease-colorimetric method.

**Assay Principle**
The reaction involved in the assay system is as follows:

\[
\text{Urea + H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2
\]

The free ammonia in an alkaline pH and in the presence of indicator forms coloured complex proportional to the urea concentration in the specimen.

**Reagents**
- **Standard urea (ST) Aqueous primary standard**
  - 50 mg/dL
  - 8.33 mmol/l
- **Reagent 1 (R1 Buffer)**
  - Phosphate buffer pH 8.0
  - 100 mmol/l
  - Sodium salicylate
  - 80 mmol/l
  - Sodium nitroprusside
  - 6.0 mmol/l
  - EDTA
  - 30.0 mmol/l

- **Reagent 2 (R2 Enzyme)**
  - Urease
  - 350000 U/l

- **Reagent 3 (R3 Alkaline Reagent)**
  - Sodium hydroxide
  - 400 mmol/l
  - Sodium hypochlorite
  - 20.0 mmol/l

**Precautions and Warnings**
- Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

**Reagent Preparation, Storage and Stability**
To prepare the working solution add the content of one vial of urease (R2) to one bottle of buffer reagent (R1).

**Stability**
- 2 months at 2-8 °C.

**Specimen Collection and Preservation**
- Urine samples are prediluted 1 : 50 with ammonium free water prior to assay.

**System Parameters**
- **Wavelength**
  - 578 nm (578-623 nm)
- **Optical path**
  - 1 cm
- **Assay type**
  - End-point
- **Direction**
  - Rate (increase)
- **Temperature**
  - 15-25 °C or 37 °C
- **Zero adjustment**
  - Against Reagent blank
- **Reagent Blank Limits**
  - Low 0.02 AU
  - High 0.2 AU
- **Sensitivity**
  - 0.6 md/dL (0.1 mmol/l)
- **Linearity**
  - 200 mg/dL (33.3 mmol/l)

**Procedure**

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working solution</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-----</td>
<td>10 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

**Deterioration**
Do not use the reagent if it is turbid. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

**Symbols in Product Labelling**
- **Xi** - Irritant
- **Use by/Expiration Date**
- **CAUTION. Consult instructions for use**
- **Manufactured by**
- **Temperature Limitation**
**Calculation**

Serum urea concentration (mg/dl) = \( \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times n \)

where \( n = 50.0 \text{ mg/dl} \) (8.33 mmol/l)

Urine urea concentration is determined by multiplying the result by the dilution factor (50).

**Urea Nitrogen:** To convert the result from urea to urea nitrogen multiply the result by 0.467.

**Quality Control**

Normal & abnormal control serum of known concentrations should be analyzed with each run.

**Performance Characteristics**

**Precision**

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>60</td>
<td>144</td>
</tr>
<tr>
<td>SD</td>
<td>1.87</td>
<td>2.1</td>
</tr>
<tr>
<td>CV%</td>
<td>3.12</td>
<td>1.46</td>
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</table>

**Run to run (Reproducibility)**

<table>
<thead>
<tr>
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<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>62</td>
<td>146</td>
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<tr>
<td>SD</td>
<td>1.92</td>
<td>2.5</td>
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<tr>
<td>CV%</td>
<td>3.25</td>
<td>1.65</td>
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**Methods Comparison**

A comparison between Spectrum Diagnostics Urea/BUN reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.97 was obtained.

**Sensitivity**

When run as recommended, the minimum detection limit of the assay is 0.6 mg/dL.

**Linearity**

The reaction is linear up to a urea concentration of (200 mg/dl) 33.3 mmol/L. Specimens showing higher concentrations should be diluted 1+2 with physiological saline and repeat the assay (result x 3).

**Interfering Substances**

**Serum, plasma**

Haemolysis
Erythrocyte contamination doesn’t elevate results.

Icterus
No significant interference.

**Lipemia**
Lipemic specimens interfere with the method of Berthiot.

**Anticoagulants**

Ammonium heparin should not be used.

Others

Ammonium ions should be avoided since it may cause erroneously elevated results. Color development in the Berthlot reaction is suppressed by amines, thiols, steroids and ascorbic acid.

**Expected Values**

**Urea (Serum)**

Adults \(< 65 \text{ years} \): 15 – 50 mg/dl  (2.5-8.33 mmol/L)

Adults \( \geq 65 \text{ years} \): \( < 70 \text{ mg/dl} \) (\( \leq 11.66 \text{ mmol/L} \))

**BUN (Serum)**

Adults \(< 65 \text{ years} \): 7 – 23.5 mg/dl

Adults \( \geq 65 \text{ years} \): 7 – 32.9 mg/dl

Children : 5 – 18 mg/dL

Urine (24) hours

Urea : 20 – 35 g/24hrs  (330-580 mmol/24hrs)

BUN : 9.3 – 16.4 g/24hrs

**Analytical Range**

0.6 – 200 mg/dL (0.1 - 33.3 mmol/L).

**Waste Disposal**

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

**References**

3. Tietz NW, ED. Clinical guide to Laboratory tests. 2ND ED.

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tbody>
<tr>
<td>321 001</td>
<td>1 x 90 ml</td>
</tr>
<tr>
<td>321 002</td>
<td>2 x 90 ml</td>
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</table>

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.