Urea/BUN – (UV)
Ultimate Single Reagent

Intended Use
Spectrum Diagnostics Ultimate urea reagent is intended for the in-vitro quantitative, diagnostic determination of urea in human serum or urine on both automated and manual applications.

Background
Urea is the major 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-UV fixed rate (enzymatic method).

Assay Principle
The series of reactions involved in the assay are as follows:

1. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide.

\[
\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\text{NH}_3 + CO_2
\]

2. In the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH), the ammonia combines with α-ketoglutarate (α-KG) to produce L-glutamate.

\[
2\text{NH}_3 + 2\alpha-KG + 2\text{NADH} \xrightarrow{\text{GLDH}} 2\text{L-Glutamate} + 2\text{NAD}^+ + H_2O
\]

The rate decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm.

Reagents
Standard urea (ST)

- BUN: 50 mg/dL
- Urea: 107 mg/dL

Reagent
- Tris Buffer (pH 8.5): 50 mmol/L
- α-Ketoglutarate: 10 mmol/L
- Urease: 8.0 K U/L
- Sodium azide: 8.0 mmol/L
- NADH: >0.20 mmol/L
- Sodium azide: 8.0 mmol/L

The reagent also contains additives required to maintain NADH in its reduced form.

For further information, refer to the Urea/Bun reagent material safety data sheet.

Reagent Preparation
Spectrum Ultimate Urea reagent is supplied ready-to-use and stable up to the expiry date labeled on the bottles. Once opened, the opened vial is stable for 3 months at 2 - 8 °C.

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 (R) contains sodium azide which may react with copper or lead plumbing.

Reagent Storage and Stability
Spectrum Urea UV Single reagent is supplied ready-to-use and stable up to the expiry date labeled on the bottles. Once opened, the opened vial is stable for 3 months at the specified temperature.

Deterioration
Do not use Spectrum Ultimate Urea reagent if it is turbid or if the absorbance of the working reagent is less than 0.9 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation
No special preparation of the patient is required. Use nonhemolysed serum or plasma only. The only acceptable anticoagulants are heparin, EDTA and fluoride. Do not use ammonium heparin plasma.

Stability:
- 7 days at 15 – 25 °C; 7 days at 2 – 8 °C;
- 1 year at -20 °C

Urine samples are precluded 1: 50 with ammonium free water prior to assay.

Stability:
- 2 days at 15 – 25 °C;
- 1 month at -20 °C

System Parameters
- Wavelength: 340 nm
- Optical path: 1 cm
- Assay type: Fixed Rate
- Direction: Decrease
- Sample: Reagent Ratio e.g.: Reagent volume 1 ml
- Sample volume 10 μL
- First read time: 30 seconds
- Delay time: 60 seconds
- Last read time: 90 seconds
- Temperature: 37 °C
- Zero adjustment: Against Air
- Reagent Blank Limits: Low 0.9 AU
- High 2.0 AU
- Sensitivity: 0.9 mg/dL (0.15 mmol/L)
- Linearity: 200 mg/dL (33.2 mmol/L)

Procedure

<table>
<thead>
<tr>
<th>Standard</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (R)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>10 μL</td>
</tr>
<tr>
<td>Specimen</td>
<td>-----</td>
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</tbody>
</table>

Mix, and after 30 seconds read the absorbance A1 of the standard or specimen. Exactly 1 minute later, read the absorbance A2 of standard or specimen.
Calculation

\[
\Delta A \text{specimen} = A1 \text{specimen} - A2 \text{specimen} \\
\Delta A \text{standard} = A1 \text{standard} - A2 \text{standard} \\
\text{Serum urea concentration (mg/dL)} = \frac{\Delta A \text{specimen}}{\Delta A \text{standard}} \times n
\]

where \( n = 107.0 \text{ mg/dL} \).

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

Quality Control

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

Performance Characteristics

Precision

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
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<tr>
<td>Mean (mg/dL)</td>
<td>45</td>
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<tr>
<td>SD</td>
<td>0.7</td>
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<tr>
<td>CV%</td>
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Run to run (Reproducibility)

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
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<tr>
<td>Mean (mg/dL)</td>
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<tr>
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<tr>
<td>CV%</td>
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Methods Comparison

A comparison between Spectrum Diagnostics Urea (UV) reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.992 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.9 mg/dL.

Linearity

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

Interfering Substances

Serum, plasma

Haemolysis

Erythrocyte contamination does not elevate results. Haemolytic specimens may cause high absorbance flagging.

Icterus

No significant interference.

Lipemia

Lipemic specimens may cause high absorbance flagging. Diluted sample treatment may be recommended.

Anticoagulants

Ammonium heparin should not be used.

Others

Ammonium ions should be avoided since it may cause erroneously elevated results.

Expected Values

| Urea (Serum) | Adults <65 years: 15-50 mg/dL (2.5-8.33 mmol/L) | Adults >65 years: <70 mg/dL (<11.66 mmol/L) |
| BUN (Serum)  | Adults <65 years: 7-23.5 mg/dL | Adults >65 years: 7-32.9 mg/dL | Children: 5-16 mg/dL |

Urine (24) hours

Urea: 20-35 g/24hrs (330-580 mmol/24hrs) BUN: 5.3-16.4 g/24hrs

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.

Analytical Range

0.9 – 200 mg/dL (0.15 - 33.2 mmol/L).

Waste Disposal

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

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

References


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<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tr>
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<tr>
<td>320 003</td>
<td>4 x 50 ml</td>
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<tr>
<td>320 004</td>
<td>2 x 100 ml</td>
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ORDERING INFORMATION

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