CLINICAL SIGNIFICANCE (1-3)
Urea is the major metabolite product of protein catabolism. The biosynthesis of urea from ammonia is exclusively carried out by hepatic enzymes. More than 90% of urea is excreted through the kidneys, with the remainder excreted through the gastrointestinal tract or skin. Blood urea concentrations can be increased by numerous factors linked to prerenal causes (increased protein catabolism, as in haemorrhage into gastrointestinal tract, shock, some chronic liver diseases) or renal/postrenal causes (acute or chronic renal diseases, postrenal obstruction to urine flow). Uremia is also increased by high-protein diet, state of dehydration, muscle wasting (as in starvation). The determination of urea rate is used together with the determination of creatinine rate to discriminate-between-prerenal (normal-creatinine) and renal/postrenal (increased creatinine) disorders.

METHOD (3-5)
Enzymatic - UV
Kinetic

PRINCIPLE (3-5)
Enzymatic determination according to the following reactions:

\[
\text{Urea} + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-}
\]

\[
\text{NH}_4^+ + \alpha\text{-Ketoglutarate} + \text{NADH} \rightarrow \text{L-Glu} + \text{NAD}^+ + \text{H}_2\text{O}
\]

\[
\text{GIDH} = \text{Glutamate dehydrogenase}
\]

REAGENTS COMPOSITION
Reagent 1: R1
Tris buffer, pH 7.60 (37 °C) 125 mmol/L
ADP 1 mmol/L
\(\alpha\)-Ketoglutarate 9 mmol/L
Urease \(\geq 8\) 100 U/L
GIDH \(\geq 1\) 350 U/L

Reagent 2: R2
NADH 1.5 mmol/L

Standard: Std
Urea 50 mg/dL
0.5 g/L
8.32 mmol/L

This standard is included in kits URSL-0407/0427/0507 and can be sold separately under the reference URUV-0055.

MAERIAL REQUIRED BUT NOT PROVIDED
CONT-0060 ELITROL I 10-5 mL
CONT-0160 ELITROL II 10-5 mL

PRECAUTIONS
- The reagents contain less than 0.1% of sodium azide. Sodium azide can react with copper and lead plumbing to form explosive metal azides. If discharge in the canalisations, rinse with plenty of water.
- Use clean or single use laboratory equipment only to avoid contaminations.
- The standard should be immediately and tightly capped to prevent contamination and evaporation.

WASTE MANAGEMENT
Disposal of all waste material should be in accordance with local and legal requirements.

STABILITY OF REAGENTS
To store at 2-8 °C and protected from light.

The reagents and the standard are stable until the expiry date stated on the label.

On board stability: The stability is specific for each analyser (for Selectra refer to § PERFORMANCE DATA)

PREPARATION AND STABILITY OF WORKING REAGENT
- The standard is ready to use
- One-reagent procedure Mix 4 volumes of reagent R1 with 1 volume of reagent R2.
Stability: 5 days at 20-25 °C
4 weeks at 2-8 °C
- Two-reagent procedure The reagents are ready to use.

SAMPLES (2,3)
- Specimen
- Serum or heparinized plasma (except ammonium heparin). Do not use fluoride as inhibitor of glycolysis since it inhibits Urease.
- Urines should be diluted at 1/20 to 1/50 with distilled water before analysis (when there is no predilution by the analyser).
- Storage Serum and plasma are stable up to 24 hours at room temperature, for one week at 4 °C. Frozen between -15 and -20 °C, these samples are stable for at least 2-3 months. Urine samples are stable up to 4 days if stored at 4-8 °C. Urines can be preserved with thymol to avoid bacterial action or by maintaining the pH below 4.

REFERENCE VALUES (1,2)
Serum: 13 - 43 mg/dL Urine: 26 - 43 g/24 h
0.13 - 0.43 g/L 430 - 710 mol/24 h
2.1 - 7.1 mmol/L

Note: It is recommended for each laboratory to establish and maintain its own reference values. The data given here are only an indication.

PROCEDURE
This reagent can be used on most analysers, semi-analysers and manual method.
The applications are available on request.
Wavelength: 340 nm
Temperature: 37 °C
Read against reagent blank.

One-reagent procedure

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Calibration</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>300 µL</td>
<td>300 µL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3 µL</td>
<td>3 µL</td>
</tr>
<tr>
<td>Standard</td>
<td>3 µL</td>
<td>3 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>3 µL</td>
<td>3 µL</td>
</tr>
</tbody>
</table>

Mix and read the variation of absorbance (ΔA) between 25 seconds and 75 seconds.

Two-reagent procedure

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Calibration</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent R1</td>
<td>200 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Reagent R2</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

Mix and wait 25 seconds.
**UREA UV SL**

*In vitro diagnostic reagent, for professional use only*

<table>
<thead>
<tr>
<th>Distilled water</th>
<th>2.5 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>2.5 µL</td>
</tr>
</tbody>
</table>

Mix and read the variation of absorbance (AA) between 25 seconds and 75 seconds.

**CALCULATION**

\[ \Delta A \text{ Sample} \times n \quad n = \text{standard concentration} \]

Take the dilution factor into account for the calculation of urea concentration in urine.

**CALIBRATION**

Concentration value of Urea Standard 50 mg/dL is traceable to the Standard Reference Material SRM 909b (of the National Institute of Standards and Technology).

The calibration frequency is specific for each analyser (for Selectra refer to § PERFORMANCE DATA).

**QUALITY CONTROL**

To ensure adequate quality, control sera such as ELITROL I (normal control) and ELITROL II (abnormal control) are recommended.

**PERFORMANCE DATA at 37 °C on Selectra**

- **Analytical range**
  - The reagent is linear from 10 to 300 mg/dL
  - Detection limit: 5.5 mg/dL (0.055 g/L, 0.92 mmol/L)
  - Analytical range: 8 mg/dL (0.080 g/L, 1.34 mmol/L)

- **Interferences**
  - Bilirubin: No significant interference up to 60 mg/dL
  - Hemoglobin: No significant interference up to 500 mg/dL
  - Ascorbic acid: No significant interference up to 23 mg/dL
  - Triglycerides: No significant interference up to 600 mg/dL
  - Methyldopa: No significant interference up to 5 mg/dL

Other compounds may interfere.

**On-board Stability/Calibration Frequency on for Selectra (no refrigerated)**

- On-board Stability: 5 days for one-reagent procedure and 14 days for two-reagent procedure. Capped vials and stored at 2-8 ºC during the night.
- Calibration Frequency: 5 days for one-reagent procedure and 14 days for two-reagent procedure.

Make a new calibration when reagent lots change, when quality control results fall outside the established range, and after a maintenance operation.

**BIBLIOGRAPHY**


**SYMBOLS USED ON LABELS**

- **IVD**: In vitro diagnostic medical device
- **M**: Consult Instruction for use
- **P**: Manufacturer’s address
- **R**: Catalogue number
- **L**: Temperature limitation
- **T**: LOT number
- **E**: Expiration date

**Revised Version: PIT-URSL-12 01/02/2010**