UREA
UREASE-BERTHELOT METHOD
COLORIMETRIC MANUAL
RX MONZA

INTENDED USE
For the quantitative in vitro determination of Urea in serum, plasma and urine. This product is suitable for manual use and on the RX Monza analyser.

Cat. No.
UR 1068 R1a. Urease 1 x 1.0 ml
170 ml R1b. Sodium nitroprusside 1 x 37 ml
R2. Phenol concentrate 1 x 110 ml
R3. Hypochlorite concentrate 1 x 22 ml
CAL Standard 1 x 5.5 ml

PRINCIPLE(2)
Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot’s reaction.

\[
\text{Urea} + H_2O \rightarrow 2\text{NH}_3 + \text{CO}_2
\]

\[
\text{NH}_3 + \text{hypochlorite} + \text{phenol} \rightarrow \text{indophenol} \quad \text{(blue compound)}
\]

SAMPLE MATERIAL
Serum, heparinized or EDTA plasma, or urine.
Dilute urine 1 + 20 in distilled water.

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>Contents</th>
<th>Initial Concentration of Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1. EDTA</td>
<td>116 mmol/l</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>6 mmol/l</td>
</tr>
<tr>
<td>Urease</td>
<td>1 g/l</td>
</tr>
<tr>
<td>R2. Phenol (diluted)</td>
<td>120 mmol/l</td>
</tr>
<tr>
<td>R3. Sodium hypochlorite (diluted)</td>
<td>27 mmol/l</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>0.14 N</td>
</tr>
<tr>
<td>CAL Standard</td>
<td>See lot specific insert</td>
</tr>
</tbody>
</table>

SAFETY PRECAUTIONS AND WARNINGS
For in vitro diagnostic use only. Do not pipette by mouth.
Exercise the normal precautions required for handling laboratory reagents.

Solution R1a contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up.
Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS
R1. Sodium nitroprusside and urease (Solution R1)
Transfer the contents of vial R1a into bottle R1b and mix gently. Stable for 2 months at +2 to +8°C.

R2. Phenol
Dilute contents of bottle R2 with 660 ml of distilled water. Rinse bottle thoroughly and mix. Store in a dark bottle. Stable for 2 months at +2 to +8°C.

R3. Sodium hypochlorite
Dilute the contents of bottle R3 with 750 ml of distilled water. Rinse bottle thoroughly and mix. Store in a dark bottle. Stable for 3 months at +2 to +8°C.

MATERIALS PROVIDED
Urease
Sodium nitroprusside
Phenol concentrate
Hypochlorite concentrate
Standard

MATERIALS REQUIRED BUT NOT PROVIDED
Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
PROCEDURE
Select URMD in the Run Test screen and carry out a water blank as instructed.

Pipette into a cuvette:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>-----</td>
<td>-----</td>
<td>5 µl</td>
</tr>
<tr>
<td>Standard (CAL)</td>
<td>-----</td>
<td>5 µl</td>
<td>-----</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>5 µl</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Mix and incubate at 37°C for 10 min.

Reagent 2

1.25 ml 1.25 ml 1.25 ml

Reagent 3

1.25 ml 1.25 ml 1.25 ml

Mix immediately and incubate at 37°C for 15 min.

Insert into the Monza flowcell folder and press read. The colour of the reaction is stable for at least 8 hours.

FOR MANUAL USE

Wavelength: 546 nm (530-570 nm)

Cuvette: 1 cm light path

Temperature: 37°C

Measurement: against reagent blank

Pipette into test tubes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>-----</td>
<td>-----</td>
<td>10 µl</td>
</tr>
<tr>
<td>Standard (CAL)</td>
<td>-----</td>
<td>10 µl</td>
<td>-----</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>10 µl</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Mix and incubate at 37°C for 10 min.

Reagent 2

2.50 ml 2.50 ml 2.50 ml

Reagent 3

2.50 ml 2.50 ml 2.50 ml

Mix immediately and incubate at 37°C for 15 min.

Read absorbance of the sample (A_sample) and standard (A_standard) against the blank. The colour of the reaction is stable for at least 8 hours.

MANUAL CALCULATION

1. Serum or Plasma

\[
\text{Urea Concentration} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard conc.} \quad (\text{mmol/l})
\]

\[
\text{Urea Concentration} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard conc.} \quad (\text{mg/dl})
\]

2. Urine

\[
\text{Urea Concentration} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard conc.} \quad (\text{mmol/l})
\]

\[
\text{Urea Concentration} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard conc.} \quad (\text{g/l})
\]

Note: 1 mg of urea corresponds to 0.467 mg of urea nitrogen.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland (028) 94422413.

INTERFERENCE

All anticoagulants except ammonia-heparinate can be used. Turbid, icteric and haemolytic serum or plasma samples should not be used as they may interfere with the assay.

NORMAL VALUES\(^{(4)}\)

<table>
<thead>
<tr>
<th></th>
<th>Serum:</th>
<th>Urine:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.7-9.1</td>
<td>333-583</td>
</tr>
<tr>
<td></td>
<td>mmol/l</td>
<td>mmol/24h</td>
</tr>
<tr>
<td></td>
<td>10-55</td>
<td>20-35</td>
</tr>
<tr>
<td></td>
<td>mg/dl</td>
<td>g/24h</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using an RX Monza analyzer in cuvette mode at 37°C.

SERUM

LINEARITY

This method is linear up to 40.0 mmol/l (241 mg/dl) in serum or plasma. Samples above this concentration should be diluted 1 + 1 with 0.9% NaCl and reassayed, multiplying the results by 2.
SENSITIVITY
The minimum detectable concentration of Urea was determined as 1.59 mmol/l (9.57 mg/dl).

PRECISION

<table>
<thead>
<tr>
<th>Within run precision</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dl)</td>
<td>49.2</td>
<td>107</td>
</tr>
<tr>
<td>SD</td>
<td>0.162</td>
<td>0.310</td>
</tr>
<tr>
<td>CV(%)</td>
<td>0.33</td>
<td>0.29</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total precision</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/dl)</td>
<td>49.2</td>
<td>107</td>
</tr>
<tr>
<td>SD</td>
<td>2.78</td>
<td>1.90</td>
</tr>
<tr>
<td>CV(%)</td>
<td>5.66</td>
<td>1.78</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

METHOD COMPARISON
The Randox method (Y) was compared to another commercially available test kit (X) and the following linear regression equation obtained:

\[ Y = 0.9437X + 1.8741 \]

and a correlation coefficient of \( r = 0.9970 \)

43 patient samples were analysed spanning the range 16 – 181 mg/dl.

URINE

LINEARITY
This method is linear up to 844 mmol/l (5075 mg/dl) in urine. Samples above this concentration should be diluted 1 + 1 with 0.9% NaCl and reassayed, multiplying the results by 2.

SENSITIVITY
The minimum detectable concentration of Urea was determined as 25.9 mmol/l (155 mg/dl).

REFERENCES