QUANTITATIVE DETERMINATION OF UREA

ORDER INFORMATION

| REF: UREN 200 Cont. 2x100 ml |

CLINICAL SIGNIFICANCE

Urea is the final result of the metabolism of proteins; it is formed in the liver from their destruction. It can appear the urea elevated in blood (uremia) in: diets with excess of proteins, renal diseases, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction1,4,5. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE

Urea condenses with o-phthalaldehyde and Naphthyl ethylene diamine to form an orange colored complex. The rate of formation of this complex is directly proportional to urea concentration, and is monitored on an initial rate (fixed time) mode at 505 nm.

REAGENT COMPOSITION

Reagent I : O-phthalaldehyde reagent
Reagent II : NED reagent
Reagent III : Urea Standard 50 mg/dL

SAFETY PRECAUTIONS AND WARNINGS

R2: Corrosive (C). R35: Causes severe burns. S26: In case of contact with eyes, rinse immediately with plenty of water and sep medical advice. S30: Never add water to this product. S45: In case of accident or if you feel unwell, sep medical advice immediately.

SAMPLE COLLECTION AND PRESERVATION

Unhemolyzed serum or plasma. The specimen is stable for 3 days when stored at 2-8°C.

REAGENT PREPARATION AND STORAGE

FOR FIX TIME KINETIC METHOD

Reagent I, II & III are ready for use as supplied.

REAGENT STABILITY

Reagents I & II are stable at room temperature (15-30°C)

LINEARITY

The method is linear to a concentration of 300 mg/dl

AUTOMATED PARAMETERS

FIX TIME, KINETIC METHOD

| Wavelength | 505 |
| React. Temp | 37°C |
| Reaction Type | Fix Time Kinetic |
| Light Path | 1 cm |
| Measurement | Against DI Water |
| Sample Volume | 25 μl |
| Reagent I Volume | 500 μl |
| Reagent II Volume | 500 μl |
| Concentration of Standard | 50 mg/dl |
| Delay Time | 60 seconds |
| Interval | 60 seconds |
| No. of Reading | 1 |
| Maximum Absorbance Limit | 2.0 |
| Normal Low | 15 mg/dl |
| Normal High | 40 mg/dl |
| Linearity | 300 mg/dl |

ASSAY PROCEDURE

FOR TWO-STEP FIX TIME KINETIC METHOD

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample</th>
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<tbody>
<tr>
<td>Sample</td>
<td>-</td>
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<tr>
<td>Standard</td>
<td>25 μl</td>
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OPA Reagent I 500 μl 500 μl

Mix Well & add 500μl of NED Reagent II to the Standard tube, When the results of the Standard have been printed, add 500μl NED Reagent II to the next tube and aspirate after mixing briefly.

Mix & after 60 Sec. read initial absorbance and start timer simultaneously. Read again after 60 sec. Determine Δ A /min. of standard (AS) and sample (AC) against distill water blank at 505 nm.

CALCULATION

Δ AC / Δ AS x C = mg urea / dl Serum

C= Concentration of standard

REAGENT PREPARATION & STORAGE

FOR SINGLE-STEP END - POINT METHOD

Just prior to use, mix one volume of Reagent I, (O-phthalaldehyde Reagent) with 1 volume of Reagent II, (NED Reagent). Mix well. Label it as “Working Urea Reagent”, prepare only quantity required for immediate use.

NOTE

The Working Urea Reagent should be used within one hour after preparation. Protect Working Reagent from high temperature and strong light at all times.
LINEARITY

The method is linear to a concentration of 300 mg/dl. Dilute the sample when the concentration of urea in the sample exceeds 300 mg/dl, multiply the results obtained by the appropriate dilution factor.

AUTOMATED PARAMETERS

END-POINT METHOD

- **Wavelength**: 505 nm
- **Reaction Temperature**: 37°C
- **Reaction Type**: End Point
- **Light Path**: 1 cm
- **Measurement**: Against Reagent Blank
- **Sample Volume**: 25 μl
- **Working Reagent Volume**: 1000 μl
- **Concentration of Standard**: 50 mg/dl
- **Incubation**: 5 mins.
- **Maximum Absorbance Limit**: 2.0
- **Normal Low**: 15 mg/dl
- **Normal High**: 40 mg/dl
- **Linearity**: 300 mg/dl

ASSAY PROCEDURE

END-POINT METHOD

<table>
<thead>
<tr>
<th></th>
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<th>Standard</th>
<th>Sample</th>
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<tr>
<td>Standard</td>
<td>-</td>
<td>25 μl</td>
<td>-</td>
</tr>
<tr>
<td>Working Reagent</td>
<td>1000 μl</td>
<td>1000 μl</td>
<td>1000 μl</td>
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</tbody>
</table>

Mix well & Incubate at 37°C for 5 mins. Read the absorbance of standard (AS) & Sample (AC) against Reagent Blank at 505 nm

CALCULATION

\[
\frac{AC}{AS} \times C = \text{mg urea/dl Serum}
\]

\[C = \text{Concentration of Standard}\]

QUALITY CONTROL

Accutestrol N - H

REFERENCE INTERVAL

15 - 40 mg/dl

INTERFERENCES

1. Bilirubin upto 10 mg/dl and hemoglobin upto 1.5 mg/dl do not effect the assay.
2. Sulfamethaxole at therapeutic level results in about 3 mg/dl increase in urea concentrations, which may be clinically significant.
3. Rarely, turbidity may appear in the assay. This may be overcome by diluting the samples with equal volumes of normal saline and multiplying the results obtained by 2.

BIBLIOGRAPHY