# UREA U.V.

## Enzymatic and colourimetric determination of Urea in biological liquids

### TEST SUMMARY

The Urea is being hydrolized by means of Urease to Ammonia, which reacts in presence of Glutamate dehydrogenase, with 2-oxoglutarate and NADH to form glutamate and $NAD^+$.
The decrease of absorbance is measured at 340 nm.

### SAMPLES

Serum, plasma (avoid ammonium heparinate).
Urine diluted 1:100 with deionized water.
Urea is stable 3 days at 2-8°C.

### REAGENTS

| | |
|---|---|
| Reagent A: | Tris pH 7.60 115 mM, ADP 1.2 mM, NADH 0.25 mM, Urease ≥ 8 KU/lt, GLDH ≥ 800U/lt, stabilizers. |
| Reagent B: | 2-oxoglutarate Sodium 7.5 mM, stabilizers. |
| Standard: | Urea 50 mg/dl; stabilizers and preservatives. |

### MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solutions.

### PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GPL) guidelines.

### REAGENTS PREPARATION

Add 20 ml of reagent B to a bottle of reagent A.
Stability: 60 days at 2-8°C.
Keep at room temperature before use.

### TEST PROCEDURE (STARTER SAMPLE)

| | |
|---|---|
| Kind of analysis: | Fixed time (decreasing) |
| Reading time: | 30, 90 seconds |
| Delay: | 30 seconds |
| Wavelenght: | 340 nm |
| Temperature: | 37°C |
| Lightpath: | 1 cm |
| Zero: | Blank reagent |

| REAGENTS | BLANK | STANDARD | SAMPLE |
|---|---|---|---|
| Working reagent | 1 ml | 1 ml | 1 ml |
| Incubate at 37 °C for 5 minutes | | | |
| Distilled water<br>Standard<br>Sample | 10 µl<br>--<br>-- | --<br>10 µl<br>-- | --<br>--<br>10 µl |
| Record the absorbance after 30 seconds (A1) at 340 nm, record again after exactly 60 seconds (A2). | | | |

### CALCULATION

**Serum/Plasma** Urea (mg/dl)

$$[A_2 - A_1\,(_{sample})] / [A_2 - A_1\,(_{standard})] \times 50$$

(standard value)

**Random urine** Urea (mg/dl)

$$[A_2 - A_1\,(_{sample})] / [A_2 - A_1\,(_{standard})] \times 50 \times 100$$

(standard value and dilution)

**24 hours urine** Urea (g/24h)

$$\frac{[A_2 - A_1\,(_{sample})] / [A_2 - A_1\,(_{standard})] \times 50 \times 100 \times \text{dl urine}}{1000}$$

(standard value, dilution factor and diuresis in decilitres)

### EXPECTED VALUES

| | | |
|---|---|---|
| Adults | 10 – 50 mg/dl | (1.7 – 8.3 mmol/l) |
| Urine | 20 – 35 g/24 h | (332 – 580 mmol/24 h) |

Each laboratory should establish appropriate reference intervals related to its population.

### NOTE

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

### CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

**QN 0050 CH** **10 x 5 ml**
Control Sera normal values

**QP 0050 CH** **10 x 5 ml**
Control Sera pathological values

### TEST PERFORMANCE

**Precision**

| Intra-assay (n = 30) | Mean (mg/dl) | SD (mg/dl) | CV% |
|---|---|---|---|
| Sample 1 | 41.23 | 0.7738 | 1.88 |
| Sample 2 | 129.7 | 0.7497 | 0.58 |

| Inter-assay (n = 30) | Mean (mg/dl) | SD (mg/dl) | CV% |
|---|---|---|---|
| Sample 1 | 40.56 | 1.3308 | 3.28 |
| Sample 2 | 130.30 | 1.2077 | 0.93 |

**Sensitivity/Limit of detection**
The method is able to discriminate until 1 mg/dl.

**Linearity**
The method is linear up to 300 mg/dl.
If the values are exceeded, it is suggested to dilute the sample 1+9 with saline and to repeat the test, multiplying the results by10.

**Methods comparison**
A comparison with a commercial available product gave the following results in a comparison on 100 samples:

Urea LTA = x
Urea competitor = y
n = 31

y = 0,98577x + 0.38516 mg/dl r = 0,9795

**Interferences**
No interference was observed by the presence of:

| | |
|---|---|
| haemoglobin | ≤ 500 mg/dl |
| bilirubin | ≤ 28 mg/dl |
| lipids | ≤ 600 mg/dl |

### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

### PACKAGING

| CODE CC02301 | (400 TESTS) | |
|---|---|---|
| Reagent A | 4 x 80 ml | (liquid) |
| Reagent B | 2 x 40 ml | (liquid) |
| Standard | 1 x 5 ml | (liquid) |

### REFERENCES

Falke, H.N. Schubert, G.E. Klin. Wschr.42 (1965).
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashood (1994).
HU Bergmeyer – Methods of enzymatic analysis, (1987).

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### SYMBOLS

| | |
|---|---|
| IVD | Only for IVD use |
| LOT | Lot of manufacturing |
| REF | Code number |
| | Storage temperature interval |
| | Expiration date (year, month) |
| | Warning, read enclosed documents |
| | Read the directions |
| | Biological risk |

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