SUMMARY
Urea constitutes the most important fraction of non-protein nitrogen present in most biological fluids. It is the main final product of protein metabolism in human beings. It is produced in the liver and is eliminated by the kidneys. Increased urea levels are associated with possible renal dysfunction. However, it should be considered that urea values are closely related to diet and protein metabolism, therefore any alteration will result in a change on urea levels.

PRINCIPLE
Ammonia produced by urea hydrolysis, catalyzed by urease, reacts with salicylate and hypochlorite in alkaline medium, producing green indophenol.

PROVIDED REAGENTS
A. Reagent A: concentrated solution containing phosphates buffer 200 mmol/l, salicylic acid 750 mmol/l, sodium nitroprusside 20 mmol/l and EDTA 10 mmol/l.
B. Reagent B: 10 mmol/l sodium hypochlorite concentrated solution in 0.1 mol/l sodium hydroxide.
C. Reagent C: urease ≥ 75 U/ml in glycerinated solution.
S. Standard: 0.60 g/l urea solution (28.4 mg/dl BUN).

NON-PROVIDED REAGENTS
Distilled water.

INSTRUCTIONS FOR USE
Reagent A preparation: dilute 1 part of concentrated Reagent A + 4 parts of distilled water.
Reagent B preparation: dilute 1 part of concentrated Reagent B + 4 parts of distilled water.
Reagent A+C: add 4 ml Reagent C to 100 ml of Reagent A. Larger volumes may be prepared maintaining the stated proportion.

WARNINGS
Reagents are for “in vitro” diagnostic use. Use the reagents according to the working procedures for clinical laboratories. The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagents: stable at 2-10°C until the expiration date stated on the box.
Reagents A and B: stable for up to 1 year from preparation stored at 2-10°C.
The mixture of Reagent A+C is stable for up to 20 days at 2-10°C from preparation.

INSTABILITY OR DETERIORATION OF REAGENTS
Blank values greater than 0.150 O.D. indicate reagent deterioration. Discard in such case.

SAMPLE
Serum, plasma or urine
a) Collection: obtain serum in the usual way.
b) Additives: for plasma, the use of Anticoagulante W from Wiener lab is recommended.
c) Known interfering substances:
- Anticoagulants containing fluorides inhibit urease’s activity.
- No interferences have been observed with slight or moderate hemolysis or with bilirubin up to 400 mg/l.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: serum urea is stable several days at 2-10°C or 6 months at -20°C without preservatives.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated values.
- Water bath at 37°C (optional).
- Stopwatch.

ASSAY CONDITIONS
- Reaction temperature: 37°C or room temperature.
- Reaction time: 10 minutes at 37°C or 20 minutes at room temperature.
- Reaction volume: 2 ml
- Sample volume: 10 ul

PROCEDURE
I- SERUM OR PLASMA TECHNIQUE
In three test tubes labeled B (Blank), S (Standard), and U (Unknown) place:

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<tr>
<th></th>
<th>B</th>
<th>S</th>
<th>U</th>
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</thead>
<tbody>
<tr>
<td>Standard</td>
<td>-</td>
<td>10 ul</td>
<td>-</td>
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</table>
Serum or Plasma 10 ul
Reagent A+C 1 ml 1 ml 1 ml
Mix. Incubate for 5 minutes at 37°C or for 10 minutes at room temperature. Then add:

Reagent B 1 ml 1 ml 1 ml
Mix. Incubate for 5 minutes at 37°C or for 10 minutes at room temperature. Measure absorbance in spectrophotometer at 570 nm or in photocolorimeter with orange filter (560-580 nm).

II- URINE TECHNIQUE
Assay is performed following the same procedures as for serum or plasma, using urine diluted with water or saline solution. Since urea contents are usually related to specific gravity, it is advisable to perform dilution according to the following data:

Specific gravity up to 1.015 ........................... dilute 1/10
Specific gravity from 1.016 to 1.025.............. dilute 1/20
Specific gravity higher than 1.025.................... dilute 1/40

As urine may contain variable amounts of ammonium, a Urine Blank (UB) should be included in each determination. Process the Blank in the same way as the Reagent Blank (B) but after Reagent B, add 10 ul of the urine dilution. Set instrument to zero O.D. with Reagent Blank (B); perform reading of Standard (S), Urine Blank (UB) and Unknown (U).

STABILITY OF FINAL REACTION
Final reaction color is stable for up to 2 hours. Therefore, absorbance reading may be performed within this period.

CALCULATIONS
Serum or plasma:
Urea (g/l) = U x factor
factor = 0.60 g/l
S

Urine
(U - UB) x 0.60 g/l
Urea g/l = x dilution
S

QUALITY CONTROL METHOD
If the sample to be tested is serum, analyze 2 levels of a quality control material (Standatrol S-E 2 niveles) with known urea concentration in each determination. If running urine samples, a urine-based control should be used.

SI SYSTEM UNITS CONVERSION
Urea (g/l) x 16.67 = Urea (mmol/l)
BUN (mg/dl) x 0.357 = Urea (mmol/l)

REFERENCE VALUES
Serum or plasma: 0.10 - 0.50 g/l
This range was obtained from samples of 120 fasting individuals from both sexes, with ages ranging from 20 and 45 years, living in Rosario (Argentina), with no symptoms of renal dysfunction or some other apparent disease.

Urine: normally, urea elimination shows great variations depending on diet. On average and with an ordinary mixed diet, 30 g are excreted in 24 hours, with oscillations ranging from 20 g to 40 g.

It is recommended that each laboratory establishes its own reference values.

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE.

PERFORMANCE
a) Reproducibility: when replicates of the same sample were simultaneously tested, the following results were obtained:

<table>
<thead>
<tr>
<th>Level</th>
<th>S.D.</th>
<th>C.V.</th>
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<tbody>
<tr>
<td>0.60 g/l</td>
<td>± 0.008 g/l</td>
<td>1.32 %</td>
</tr>
<tr>
<td>2.28 g/l</td>
<td>± 0.045 g/l</td>
<td>1.97 %</td>
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b) Linearity: reaction is linear up to 2.50 g/l. If absorbance readings results higher in the instrument employed, may be used 1.5 ml of each Reagent and 10 ul sample to obtain the desired linearity.

When urea concentration exceeds 2.50 g/l or is above the linearity of the instrument, may be diluted the final reaction with the blank. Under these conditions it is linear up to 5 g/l.

b) Recovery: a recovery between 94.2% and 100.6% was obtained by adding known amounts of urea to different aliquots of the same sample.

c) Analytical sensitivity: depends on the photometer used and on the wavelength. In spectrophotometers with 1 cm width square cuvettes, for ∆A of 0.001 the minimum detectable concentration change will be 0.0125 g/l.

WIENER LAB. PROVIDES
Kit for 500 determinations (Cat N° 1810050).

REFERENCES
Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

- **CE**
  - This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

- **EC REP**
  - Authorized representative in the European Community

- **IVD**
  - "In vitro" diagnostic medical device

- **Σ**
  - Contains sufficient for <n> tests

- **Time**
  - Use by

- **Temperature limitation**
  - Temperature limitation (store at)

- **Do not freeze**

- **Biological risks**

- **Volume after reconstitution**

- **Contents**

- **Lot**
  - Batch code

- **Calibr.**
  - Calibrator

- **Control**

- **Positive Control**

- **Negative Control**

- **Harmful**

- **Corrosive / Caustic**

- **Irritant**

- **Consult instructions for use**

- **Manufactured by:**

- **Authorized representative in the European Community**

- **Volume after reconstitution**

- **Contents**

- **Batch code**

- **Catalog number**