COD 11516 4 x 50 mL	COD 11517 2 x 250 mL	COD 11541 1 x 1 L
STORE AT 2-8ºC		
Reagents for measurement of urea concentration Only for *in vitro* use in the clinical laboratory		

UREA/BUN - UV

BioSystems

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UREA/BUN - UV
UREASE / GLUTAMATE DEHYDROGENASE

PRINCIPLE OF THE METHOD

Urea in the sample consumes, by means of the coupled reactions described below, NADH that can be measured by spectrophotometry[1,2].

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2 – oxoglutarate} \xrightarrow{\text{glutamate dehydrogenase}} \text{Glutamate} + NAD^+$$

CONTENTS

	COD 11516	COD 11517	COD 11541
A. Reagent	4 x 40 mL	2 x 200 mL	1 x 800 mL
B. Reagent	4 x 10 mL	2 x 50 mL	1 x 200 mL
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Tris 100 mmol/L, 2-oxoglutarate 5.6 mmol/L, urease > 140 U/mL, glutamate dehydrogenase > 140 U/mL, ethyleneglycol 220 g/L, sodium azide 0.95 g/L, pH 8.0.

B. Reagent. NADH 1.5 mmol/L, sodium azide 9.5 g/L.
WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL, urea 50 mg/dL (8.3 mmol/L, BUN 23.3 mg/dL), creatinine 2 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank lower than 1.100 at 340 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

REAGENT PREPARATION

Working Reagent: Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC
- Analyzer, spectrophotometer or photometer able to read at 340 nm.

SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/50 with distilled water before measurement.

Urea in serum or plasma is stable for 7 days at 2-8ºC. Heparin is recommended as anticoagulant[3].

Urea in urine is stable for 2 days at room temperature if microbial growth is prevented[3].

PROCEDURE

1. Bring the Working Reagent and the photometer to 37ºC.
2. Pipette into a cuvette (Note 1):

Working Reagent	1.5 mL
Standard (S) or Sample	10 µL

3. Mix and insert the cuvette into the photometer. Start stopwatch.
4. Record the absorbance at 340 nm after 30 seconds (A_1) and after 90 seconds (A_2).

CALCULATIONS

The urea concentration in the sample is calculated using the following general formula:

$$\frac{(A_1\text{-}A_2)_{\text{Sample}}}{(A_1\text{-}A_2)_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} = C_{\text{Sample}}$$

If the Urea Standard provided has been used to calibrate (Note 2):

	Serum and plasma	Urine
$\frac{(A_1\text{-}A_2)_{\text{Sample}}}{(A_1\text{-}A_2)_{\text{Standard}}}$	x 50 = mg/dL urea x 23.3 = mg/dL BUN x 8.3 = mmol/L urea	x 2500 = mg/dL urea x 1165 = mg/dL BUN x 415 = mmol/L urea

REFERENCE VALUES

Serum and plasma[4]: 12.8 - 42.8 mg/dL urea = 6 - 20 mg/dL BUN = 2.14 - 7.14 mmol/L urea. Concentrations in the neonatal period are lower, and in adults over 60 years of age are higher than in adults. Concentrations also tend to be slightly higher in males than in females.

Urine[3]: 26 - 43 g/24-h urea = 12 - 20 g/24 h BUN = 428 - 714 mmol/24-h urea.

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, cod. 18009 and cod. 18042), level II (cod. 18007, cod. 18010 and cod. 18043) and the Biochemistry Control Urine (cod. 18054 and cod. 18066) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 2.5 mg/dL urea = 1.17 mg/dL BUN = 0.42 mmol/L urea.
- Linearity limit: 300 mg/dL urea = 140 mg/dL BUN = 50 mmol/L urea. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean urea concentration	CV	n
42 mg/dL = 7.0 mmol/L	3.3 %	20
137 mg/dL = 22.7 mmol/L	1.9 %	20

- Reproducibility (run to run):

Mean urea concentration	CV	n
42 mg/dL = 7.0 mmol/L	4.3 %	25
137 mg/dL = 22.7 mmol/L	2.8 %	25

- Sensitivity: 1.8 mΔA·dL/mg = 10.8 mΔA·L/mmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemolysis (hemoglobin 5 g/L) and elevated ammonia interfere. Other drugs and substances may interfere[5].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Urea is synthesized in the liver as a by-product of the deamination of amino acids. Its elimination in the urine represents the major route for nitrogen excretion.

Elevated urea concentration in plasma is found as a result of a high-protein diet, increased protein catabolism, after a gastrointestinal hemorrhage, mild dehydration, shock and heart failure or treatment with glucocorticoids (pre-renal uremia)[4,6].

Post-renal uremia is caused by conditions that obstruct urine outflow: nephrolithiasis, tumor or prostatic hypertrophy. The usefulness of urea as an indicator of renal function is limited by the variability of its plasma concentration as a result of nonrenal factors[4,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

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