COD 11536 4 x 50 mL	COD 11537 2 x 250 mL
STORE AT 2-8°C	
Reagents for measurement of urea concentration Only for *in vitro* use in the clinical laboratory	

UREA/BUN - COLOR

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BioSystems
REAGENTS & INSTRUMENTS

UREA/BUN - COLOR
UREASE/SALICYLATE

PRINCIPLE OF THE METHOD

Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2,3].

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

$$NH_4^+ + \text{Salicylate} + \text{NaClO} \xrightarrow{\text{nitroprusside}} \text{Indophenol}$$

CONTENTS

	COD 11536	COD 11537
A1. Reagent	2 x 48 mL	1 x 240 mL
A2. Reagent	2 x 2 mL	1 x 10 mL
B. Reagent	2 x 50 mL	1 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL

COMPOSITION

A1. Reagent: Sodium salicylate 62 mmol/L, sodium nitroprusside 3.4 mmol/L, phosphate buffer 20 mmol/L, pH 6.9.

A2. Reagent: Urease > 500 U/mL

B. Reagent Sodium hypochlorite 7 mmol/L, sodium hydroxide 150 mmol/L.
Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

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 2-8°C.

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 over 0.250 at 600 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent (B) and Standard (S) are provided ready to use.

Reagent (A): Transfer the contents of one Reagent A2 vial into a Reagent A1 bottle (Note 1). Mix thoroughly. Other volumes can be prepared in the proportion: 1 mL Reagent A2 + 24 mL Reagent A1. Stable for 2 months at 2-8°C (Note 2).

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C
- Analyzer, spectrophotometer or photometer able to read at 600 ± 20 nm.

SAMPLES

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

Urea in serum or plasma is stable for 7 days at 2-8°C. Heparin is recommended as anticoagulant.

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

PROCEDURE

1. Bring the Reagents to room temperature.
2. Pipette into labelled test tubes:

	Blank	Standard	Sample
Urea Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Pipette:

Reagent (B)	1.0 mL	1.0 mL	1.0 mL

5. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
6. Read the absorbance (A) of the Standard and the Sample at 600 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the Urea Standard provided has been used to calibrate (Note 3):

	Serum and plasma	Urine
$\frac{A_{Sample}}{A_{Standard}}$	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]: 15-39 mg/dL urea = 7-18 mg/dL BUN = 2.5-6.5 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[4]: 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, 18009 and 18042) and II (cod. 18007, 18010 and 18043) 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 1.3 mg/dL urea = 0.60 mg/dL BUN = 0.21 mmol/L urea
- Linearity limit: 300 mg/dL = 140 mg/dL BUN = 50 mmol/L urea. For higher values dilute sample 1/5 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean urea concentration	CV	n
26 mg/dL = 4.3 mmol/L	1.6 %	20
86 mg/dL = 14.2 mmol/L	0.8 %	20

- Reproducibility (run to run):

Mean urea concentration	CV	n
26 mg/dL = 4.3 mmol/L	2.4 %	25
86 mg/dL = 14.2 mmol/L	1.3 %	25

- Sensitivity: 8.6 mA·dL/mg = 0.143 mA·L/mmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). 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 2 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. It is advisable to wash the Reagent A2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.
2. The stability of Reagent A may be drastically reduced when it is not stored at 2-8°C.
3. 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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