UREA Berthelot CE

REF 1156010	REF 1156015
2 x 50 mL	4 x 100 mL
CONTENTS	**CONTENTS**
R1. Reagent 1 x 2 mL	R1. Reagent 2 x 4 mL
R2. Reagent 1 x 48 mL	R2. Reagent 2 x 96 mL
R3. Reagent 1 x 50 mL	R3. Reagent 2 x 100 mL
CAL.Standard 1 x 3 mL	CAL.Standard 1 x 3 mL
For *in vitro* diagnostic use only	

UREA

Urease/Salycilate

Enzymatic colorimetric method

ENDPOINT

PRINCIPLE

Urea is hydrolyzed by urease[1,2] into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green cromophore. The intensity of the color formed is proportional to the concentration of urea in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{UREASE}} 2\,NH_3 + CO_2$$

$$NH_4^+ + \text{Salycilate} + \text{NaClO} \xrightarrow[OH^-]{\text{NITROPRUSSIDE}} \text{Indophenol} + \text{NaCl}$$

REAGENT COMPOSITION

R1 **Enzyme reagent**. Urease > 500 U/mL. Stabilizers.

R2 **Buffered chromogen**. Phosphate buffer 20 mmol/L pH 6.9, EDTA 2 mmol/L, sodium salycilate 60 mmol/L, sodium nitroprusside 3.4 mmol/L.

R3 **Alkaline hypochlorite**. Sodium hypochlorite 10 mmol/L, NaOH 150 mmol/L.

CAL **Urea standard.** Urea 50 mg/dL (8.3 mmol/L)
Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 909b.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 600 nm > 0.110 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 1 volume of **R1** + 24 volumes of **R2**. Stable for 4 weeks at 2-8ºC and for 7 days at 15-25ºC.

SAMPLES

Serum or heparinized plasma free of hemolysis and urine (see Notes). Other anticoagulants (ammonium heparinate or double oxalate of potassium and ammonium) must not be used.
Urea in serum, plasma or urine is stable 7 days at 2-8ºC. Freeze for longer storage.

INTERFERENCES

- Lipemia (intralipid 20 g/L) does not interfere.
- Bilirubin (40 mg/dL) does not interfere
- Hemoglobin (>2 g/L) may affect the results.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbances at 600 ± 10 nm.
- Constant temperature incubator set at 37ºC.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into a cuvette:

TUBES	Blank	Sample	CAL.Standard
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
CAL.Standard	–	–	10 µL

3. Mix and incubate for 5 minutes at 37ºC or for 10 minutes at room temperature (16-25ºC).
4. Pipette:

R3	1.0 mL	1.0 mL	1.0 mL

5. Mix thoroughly and incubate the tubes for 5 minutes at 37ºC or for 10 minutes at room temperature (16-25ºC).
5. Read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank.

The color is stable for at least 2 hours protected from light.

CALCULATIONS

Serum, plasma

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL urea}$$

Samples with concentrations higher than 300 mg/dL (50 mmol/L) should be diluted 1:5 with saline and assayed again. Multiply the results by 5.

LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN
Telf. (+34) 934 694 990 Fax. (+34) 934 693 435. website www.linear.es

Urine

Dilute the sample 1:50 with distilled water and multiply the result by 50.

If results are to be expressed as SI units apply:
mg/dL x 0.1665 = mmol/L

To convert urea mass units to those of urea nitrogen apply:
mg/dL x 0.467 = mg/dL BUN

REFERENCE VALUES [5]

Serum, plasma

Newborns (< 10 days)	6.4 - 53.5 mg/dL (1.1 - 9.0 mmol/L)
Adults (12-60 years)	15 - 40 mg/dL (2.5 - 6.6 mmol/L)

In adults over 60 years of age, the reference interval is 17-50 mg/dL (2.8-8.3 mmol/L) and the concentrations tend to be slightly higher in males than in females.

Urine

Adults (normal diet)	26 - 43 g/24-h (428 - 714 mmol/24-h)

A high-protein diet causes significant increases in plasma urea concentrations and urinary excretion.
It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **BC600** HUMAN MULTISERA NORMAL
Borderline level of urea. Assayed.

REF **BC650** HUMAN MULTISERA ABNORMAL
Elevated level of urea. Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Urea is the chief end product of protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function.

Azotemia (an abnormal increase in plasma urea level) is seen mainly in renal disorders, dehydration, increase protein catabolism, high-protein diets, or gastrointestinal hemorrhage. There are two types of azotemia. The first, *prerenal azotemia*, is caused by impaired perfusion of the Kidneys due to decreased cardiac output or for any of the former causes. The second, *postrenal azotemia*, is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinary tract.
The clinical significance of the urea level in plasma is usually determined in conjugation with the plasma creatinine level. In prerenal azotemia, an increase in the plasma urea level is usually associated with a normal plasma creatinine level, where as in postrenal azotemia, there is an increase in both the urea and the plasma creatinine levels. A decrease in the urea plasma level may be associated with acute dehydration, malnutrition, and pregnancy.

NOTES

- Collect a 24-hour urine specimen into a plastic bottle free of preservatives. Keep the sample refrigerated to minimize urea hydrolysis by microorganisms or other agents.
- This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 4.79 mg/dL
- ***Linearity :*** Up to 300 mg/dL
- ***Precision:***

mg/dL	Within-run		Between-run	
Mean	62.3	141.06	62.3	141.06
SD	2.18	5.76	2.91	5.86
CV%	3.33	4.28	4.68	4.16
N	10	10	10	10

- ***Sensitivity :*** 8.900 mA/min / mg/dL Urea.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 50 r = 0.99 y = 0.923x + 0.4987

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

REFERENCES

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