ARENA BioScien

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UREA/BUN – UV

Diagnostic reagent for the in-vitro quantitative determination of urea in human serum, plasma and urine on both manual and automated systems.

REF: BS.1/UU02.050.0100	100 test	REF: BS.1/UU02.125.0250	250 test
REF: BS.1/UU02.100.0200	200 test		

CLINICAL SIGNIFICANCE

Urea is the major end product of protein nitrogen metabolism. It is synthesized by a series of reactions in the liver called the urea cycle. In the urea cycle, ammonia is converted to urea, which is carried by blood to the kidneys for elimination from the body. The circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur due to renal impairment or in some diseases such as congestive heart failure diabetes, infection, or during different liver diseases. Determination of blood urea nitrogen (BUN) is the most widely used screening test for renal function together with serum creatinine. Serum creatinine is another metabolic waste product freely filtered by the glomerulus, but does not undergo tubular reabsorption. Its steady rate of elimination is frequently used to generate an index or ratio with BUN values for normalized evaluations.

METHOD PRINCIPLE

Urea in the sample is hydrolized enzymatically into ammonia (NH_3) and carbon dioxide (CO_2).
Ammonia ions formed react with α -ketoglutarate in a reaction catalysed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD+:

$$\text{Urea} + H_2O + 2\,H^+ \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$2NH_3 + \alpha\text{-Ketoglutarate} + NADH_2 \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

The rate decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm.

REAGENT COMPOSITION

R1: Standard	
UREA	50 mg/dl
BUN	23.4 mg/dl
R2: BUFFER REAGENT	
Tris Buffer (pH 8.5)	50 mmol/L
a-Ketoglutarate	10 mmol/L
GLDH	8 KU/L
Urease	5 KU/L
Sodium azide	8 mmol/L
R3: STARTER REAGENT	
Sodium azide	> 0.20 mmol/L
NADH	8 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.
Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the UREA/BUN reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien working solution is prepared by mixing 4 volumes of R2: Buffer and 1 volume of R3: Starter eg. 400 µl R2 +100 µl R3.
Urea/BUN reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C.
Working solution is stable for 1 month at 2 – 8 °C
or 8 days at 15 – 25 °C.

Deterioration
The ***BioScien*** Urea/BUN reagent is normally clear, reagent turbidity or control values out of the assigned range may be an indication of reagent deterioration.

SPECIMEN COLLECTION AND PRESERVATION

Serum or plasma
No special preparation of the patient is required. Ensure non haemolyzed serum or plasma are used. The only acceptable anticoagulants are heparin, EDTA and fluoride, avoid ammonium which interfere with the assay.
Stability:1 days at 15 -25°C;7 days at 2 -8°C;1 month frozen at -25°C.

Urine
Urine samples are prediluted 1: 50 with ammonium free water prior to assay.
Stability: 1 days at 15 -25°C; 7 days at 2 -8°C; 1 month frozen at -25°C.

SYSTEM PARAMETERS

Wavelength	340 nm
Optical path	1 cm
Assay type	Fixed Rate
Direction	Decrease
Sample Reagent Ratio	1:100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
Test reading time	90 seconds
First read time	30 seconds
delay time	60 seconds
last read time	90 seconds
Temperature	37˚C
Incubation time	zero
Zero adjustment	Against air

Reagent Blank Limits	Low 1.00 AU High 2.00 AU
Sensitivity	0.9 mg/dL (0.15 mmol/L)
Linearity	200 mg/dL (33.2 mmol/L)

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Standard	*Specimen*
Working Solution	1.0 ml	1.0 ml
Standard	10 µl	
Specimen		10 µl

Mix, and after 30 seconds read the absorbance A1 of the standard or specimen. Exactly 1 minute later, read the absorbance A2 of standard or specimen.

CALCULATION

Serum or Plasma:

concentration (mg/dl) = $\frac{\text{(A2-A1) specimen}}{\text{(A2-A1) standard}} \times n$

where n = 107 mg/dl

Urine urea concentration is determined by multiplying the result by the dilution factor (50).

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	45	150	47	153
SD.	0.7	2.7	0.82	2.81
CV. %	1.5	1.95	1.63	2.15

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from ***BioScien*** Urea/BUN reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.9.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.9 mg/dL (0.15 mmol/L).

Linearity

The reaction is linear up to concentration of 200 mg/dl (33.2mmol/L). Specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

INTERFERING SUBSTANCES

Haemolysis

No significant interference from Erythrocyte contamination.

Icterus

No significant interference.

lipemia

Lipemic specimens interfere with the method of Berthelot.

Others

Ammonium heparin should not be used as anticoagulants. Ammonium ions should be avoided since it may cause erroneously elevated results. Color development in the Berthelot reaction is suppressed by amines, thiols, steroids and ascorbic acid.

Reducing Substances

Color development in the Berthelot reaction is suppressed by amines, thiols, steroids and ascorbic acid.

EXPECTED VALUES

Serum and plasma	***mg/dl***	***[mmol/L]***
Urea:		
Children	11-39	[1.8-6.4]
Adults < 65 years	15 -50	[2.5-8.33]
Adults > 65 years	≤ 70	[≤ 11.66]
BUN		
Children	5-18	[0.84-2.99]
Adults < 65 years	7-23.5	[1.16-3.89]
Adults > 65 years	7-32.9	[< 5.44]
Urine	***g/24hrs***	***[mmol/24hrs]***
Urea	20-35	20-35
BUN	[330-580]	[330-580]

DYNAMIC RANGE

0.9 - 200 mg/dl (0.15 – 33.2 mmol/L).

REFERENCES

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3. Shephard MD, Mezzachi RD: Clin Biochem Revs, 4:61-7, 1983.
4. Laboratory reference values. Urea nitrogen (BUN). Rochester, Minn.: Mayo Foundation for Medical Education and Research; Nov. 2010.
5. Tiffany to, jansen JM, Burtis CA,Overton JB, Scott CD. Enzymatic Kinetic Rate and end Point analyses of Substrate, By USE of A Gemsaec fast analyzer. ClinChem. 1972; 18:829-840.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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CE