UREA/BUN – Colorimetric

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

REF:V/UR02.050	100 test	REF:V/UR02.125	250 test
REF:V/UR02.100	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 (2)

The enzymatic and colorimetric method based on the hydrolyzes of urea in the presence of water and urease to produce ammonia and carbon dioxide.

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

The free ammonia in an alkaline pH and in the presence of indicator forms colored complex proportional to the urea concentration in the specimen

REAGENT COMPOSITION

Urea standard (R1)	50 mg/dl (8.33mmol/L)
Reagent (R2): (Enzyme) Urease	 >6000 U/l
Reagent (R3): (Buffer) Phosphate Buffer pH 8.0 Sodium salicylate Sodium nitroprusside EDTA	 100 mmol/L 80 mmol/L 6.0 mmol/L 30 mmol/L
Reagent (R4): (Alkaline Reagent) Sodium hydroxide Sodium hypochlorite	 400 mmol/L 20.0 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R4) contains concentrated base which is classified as Irritant substance (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.

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 **Lab.Vie**. Urea/BUN reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. 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.
NB: For mega labs having high numbers of patient specimens, working buffer reagent can be prepared (add 5ml enzyme reagent R2 to 100ml buffer reagent R3). Working buffer is stable for 1 week at the specified temperature.

Deterioration
The **Lab.Vie**.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 (5)

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	578 nm (578 – 632 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:125 1250 µl 10 µl
Temperature	37 °C or 20 - 25°C
Incubation time	5 min. at 20-25°C or 3 min. at 37°C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.02 AU High 0.2 AU
Sensitivity	0.6 mg/dL (0.1 mmol/L)
Linearity	200 mg/dL (33.3 mmol/L)

EQUIPMENT REQUIRED NOT PROVIDED

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

ASSAY PROCEDURE

Procedure 1	Blank	Standard	Specimen
R3 (Buffer)	1.0 ml	1.0 ml	1.0 ml
R2 (Enzyme)	one drop (50 µl)	one drop (50 µl)	one drop (50 µl)
Standard		10 µl	
Specimen			10 µl
Mix and incubate for at least 5 min. at 20-25°C or 3 min. at 37°C.			
R4 (Alkaline)	200 µl	200 µl	200 µl

Procedure 2 (working buffer)	Blank	Standard	Specimen
Working solution	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Specimen			10 µl
Mix and incubate for at least 5 min. at 20-25°C or 3 min. at 37°C.			
R4 (Alkaline)	200 µl	200 µl	200 µl

Mix and incubate for 5 minutes at 37°C or 10 minutes at 20-25°C. Measure absorbance of specimen "A" specimen and standard "A" standard against reagent blank.

CALCULATION

$$\text{Serum Urea concentration (mg/dl)} = \frac{(\text{A specimen}) \times n}{(\text{A standard})}$$

Where n = 50.0 mg/dl (8.33 mmol/l)
Urine urea concentration is determined by multiplying the result by the dilution factor (50).

Urea Nitrogen: To convert the result from urea to urea nitrogen multiply the result by 0.467.

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 **Lab.Vie**. 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	60	144	62	146
SD. mg/dl	1.87	2.1	1.92	2.5
CV. %	3.12	1.46	3.25	1.65

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

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**. Urea/BUN reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.97.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 0.6 mg/dl.

Linearity
The reaction is linear up to Urea/BUN concentration of 200 mg/dl (33.3 mmol/L); specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

INTERFERING SUBSTANCES (3)

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

EXPECTED VALUES (4)

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	[330-580]
BUN	9.3-16.4	[154-271]

DYNAMIC RANGE

0.6 - 200 mg/dl (0.1 - 33.3 mmol/L).

REFERENCES

1. Tietz N. W., textbook of clinical chemistry. Burtis CA, Ashwood ER, Saunders W.B. 3rd Edition, 1999 p 1239-1241
2. Patton, C. J., Crouch, S. R., Anal. Chem., 49, 464-469 (1977)
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
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.

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