UREA (BUN)

Diagnostic reagent for determination of Urea concentration.
Liquid. Dual Reagents.Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-292	100 mL
MH-293	75 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is used for the quantitative determination of urea in serum, plasma and urine.

GENERAL INFORMATION

Urea (CO[NH2]2, MA 60 Da) is the major nitrogen-containing metabolic product of protein catabolism in humans and accounts for more than 75% of non-protein nitrogen excreted from the body. Nitrogen derived from amino acids in the process of protein catabolism enters the urea cycle via intermediates including aspartate and ammonia. Urea biosynthesis is only mediated in the liver by enzymes of the urea cycle. More than 90% of urea excretion from the body occurs through the kidney, the rest through the gastrointestinal tract and skin. Blood and serum urea measurements have been used for many years as an indicator of renal function. However, it is generally accepted that creatinine measurement provides better information. Serum and urine urea measurement can still provide useful clinical information in certain situations. For example, serum urea measurement may be a useful indicator of renal function in patients with muscle wasting disorders where serum creatinine concentration is likely to be misleading. Urea measurement in dialysis fluids is widely used to assess the adequacy of renal replacement therapy.[1]

The concentration of urea in serum is affected by the speed at which it is produced and removed. While this limits its value as a test of renal function, it allows it to be used for a number of other factors. For example, a high-protein diet, increased endogenous protein catabolism, reabsorption of blood proteins after gastrointestinal bleeding, and treatment with cortisol or its synthetic analogs lead to increased urea production and hence plasma concentration; these possibilities should be considered when there is an unexplained high serum urea concentration.

Decrease in glomerular filtration rate (GFR) due to renal dysfunction of prerenal, renal or postrenal origin may lead to a decrease in urea excretion from the circulation and consequently an increase in blood urea concentration. Variable responses between the increase in blood urea concentration and blood creatinine concentration may provide useful diagnostic information.[1]

The main causes of increased blood urea concentrations as a result of renal dysfunction of postrenal origin are benign prostatic hypertrophy that obstructs urine outflow, stenosis and obstruction due to malignancy, and stone formation. In obstructive postrenal conditions such as malignancy, nephrolithiasis and prostatism, both serum creatinine and urea concentrations will increase, but the increase in serum urea is greater than that of creatinine due to increased back diffusion. This leads to the main recommended clinical use of the serum urea concentration, i.e. measuring it in conjunction with the serum creatinine concentration and then calculating the serum urea/creatinine ratio.[2]

To make a rough distinction between prerenal and intrinsic azotemia, it is also recommended to evaluate the serum urea/creatinine ratio. The reference range for this ratio for a healthy individual on a normal diet is approximately 49 to 81 mmol urea/mmol creatinine (0.049 and 0.081 mmol urea/µmol creatinine; 12 and 20 mg urea/mg creatinine). In a known case of acute renal failure (ARF), an increased urea/creatinine ratio (>81 mmol/mmol; >20 mg/mg) has been said to indicate a more prerenal cause than an intrinsic renal cause such as acute tubular necrosis.[3] This is because urea resorption from urine is higher at slow flow rates due to decreased renal perfusion and lower in the setting of tubular damage. However, a recent review found this inference to be incorrect in many cases, yet ARF patients with a urea/creatinine ratio greater than 80 mmol/mmol (20mg/mg) had a higher in-hospital mortality rate.[3]
One of the reasons that increase blood urea concentration as a result of renal dysfunction of prerenal origin is shock as a result of water and electrolyte loss due to burns, bleeding or severe diarrhea. This increases tubular reabsorption of urea and increases blood urea levels more than blood creatinine levels. Increased urea synthesis in the liver after a high protein diet, increased protein catabolism after trauma, major surgical interventions and excessive fasting, increased protein concentration in the blood and decreased extracellular fluid as a result of upper gastrointestinal system bleeding, decreased renal perfusion as a result of heart failure and hypoproteinemia are other reasons that increase urea concentration in the blood due to renal dysfunction of prerenal origin.[4]

Another index proposed to differentiate ARF of prerenal origin from those of intrinsic origin is fractional excretion of urea (FeUr):

FeUr = [urinary urea / serum urea] / [urinary creatinine / serum creatinine] ×100

In the case of prerenal acute kidney injury (AKI), vasopressin release leads to increased water and urea resorption, reducing the fraction of filtered urea excreted. A FeUr below 35% suggests prerenal causes for AKI. Provided the same units are used for serum and urine measurements of each analyte, the units do not affect the calculation. However, claims regarding the utility of this test are questioned.[1,5]

Increased urea is observed in the blood due to chronic heart failure and the urea/creatinine ratio also increases. However, an increase in the ratio has a poor prognostic value.[6] The observed increase in urea is more than expected only due to decreased renal filtration and is thought to be mediated by neurohormonal activation.[7]

Urea is commonly used as part of the clinical risk prediction score, CURB-65 (consisting of confusion, urea, respiratory rate, blood pressure and an assessment of greater than/less than 65/year), to classify patients admitted to hospital with pneumonia.[8]

Renal dysfunctions of renal origin that increase blood urea concentration are acute or chronic kidney diseases that decrease renal glomerular filtration.[4]

One of the causes of lower blood urea concentrations is starvation or malabsorption resulting in insufficient amino acids required for deamination and a consequent decrease in urea synthesis in the liver, resulting in lower blood urea concentrations. However, in extreme starvation, plasma urea may increase as increased muscle protein breakdown is the main fuel source. In patients with severe liver disease (usually chronic), urea synthesis may be impaired, leading to a decrease in plasma urea. Plasma urea concentration may also decrease as a result of water retention associated with inappropriate vasopressin secretion or dilution of plasma with IV fluids.[4] Another reason that decreases plasma urea levels is a low protein diet.[1]
Due to tubular back diffusion, measured urea clearance results in an underestimation of GFR, making it a poor clinical tool for this purpose. Previously, measurements of mean urea clearance and creatinine clearance, which under- and overestimate the actual GFR, respectively, were proposed as a routine tool for GFR estimation, but this practice is no longer recommended due to poor performance.[9] Urine urea measurement in timed samples provides a crude index of overall nitrogen balance and can be used to guide protein requirements in critically ill patients and patients receiving parenteral nutrition.[10,11]

TEST PROCEDURE

Kinetic measurement

The enzymatic reactions in the kinetic measurement are catalyzed by urease and glutamate dehydrogenase.

In the first reaction, urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2\ H_2O \xrightarrow{urease} 2\ NH_4^+ + CO_3^{2-}$$

In the second reaction, 2-oxoglutarate reacts with ammonium to produce L-glutamate in the presence of glutamate dehydrogenase (GLDH) and NADH as a coenzyme. In this reaction, two moles of NADH are oxidized to NAD^+ for every mole of urea hydrolyzed.

$$NH_{4+} + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{GLDH} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in NADH concentration is directly proportional to the concentration of urea in the sample. The decrease in absorbance is measured photometrically at a wavelength of 340 nm.

REAGENT COMPONENTS

Tris buffer	:≤ 120 mmol/L
2-Oxoglutarate	:≤ 8 mmol/L
ADP	:≤ 1.6 mmol/L
Urease	:> 300 µkat/L
GLDH	:> 800 U/L
NADH	:≤ 0.60 mmol/L
Stabilizers	

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 60 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[12]

SAMPLE REQUIREMENTS

Serum, plasma and urine can be used and are collected according to the standard procedures. Li heparinised specimen collection tubes should be preferred for plasma. It is recommended to preserve urine samples with thymol to prevent bacterial activity.

Urea activity stability in serum and plasma[23]:

7 days at +20/+25°C
7 days at +2/+8°C
1 year at -20°C

Urea activity stability in urine[23]:

2 days +20/+25ºC
7days +2/+8ºC
1 month -20ºC

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator.

Arcal Auto Calibrator (Lyophilized)
Ref.No: VT-003

Calibration stability is 60 days .Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used..

Traceability is provided by the material with reference number NIST SRM 912.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control (Lyophilized)
Ref.No: VT-001

Arcon P Level 2 Control (Lyophilized)
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum / Plasma	: 10 - 50 mg/dL
Urine / 24h	: 20 - 35 g/24h

To convert results from mg/dL to g/day;
24-hour excretion = [(V × c) ÷ 100 000] g/day

Where:
V = 24-hour urine volume (mL)
c = analyte concentration (mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.
Reference interval data have been verified by using CLSI EP28-A3c protocol.[13]

Unit Conversion:
mg/dL urea x 0.1665= mmol/L urea
mg/dL urea × 0.467 = mg/urea nitrogen

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, “Measuring Interval” refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte’s lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[14]

The determined analytic measuring interval for Urea is 1-300 mg/dL.

Detection Capability

Limit of Detection (LoD): 0.2 mg/dL

Limit of Quantitation (LoQ): 1 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[15]

Linearity

This method shows measurement linearity in the activities up to 300 mg/dL. Autoanaylzer’s auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[16]

Precision

Running system has been developed according to 20x2x2 “The Single Site” protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[17]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Urea have been given in the table 1 and 2 respectively.

Table 1. Urea Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
30.6 mg/dL	0.55	1.81	80
97.9 mg/dL	0.56	0.57	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[18]

Table 2. Urea Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
30.6 mg/dL	0.71	2.31	80
97.9 mg/dL	2.70	2.76	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[18]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[19]
y= 1.048x - 0.79 mg/dL
r=0.986

Interference

Endogenous interferant and analyte concentrations that have been used in the Urea scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[20,21]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Urea interference scanning test is appropriate, is determined as ±10%.[22]

In Urea test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Urea Target (mg/dL)	N*	Observed Recovery %
Hemoglobin 540 mg/dL	27.10	3	110
Bilirubin 48.3 mg/dL	25.37	3	109
Lipemi 570 mg/dL	27.49	3	104

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[21]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[21]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OHSAS(Occupational Health and Safety Assesment Series) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests