BECKMAN COULTER

AU/DxC AU US

Instructions For Use

UREA
UREA NITROGEN

REF OSR6134 4 x 25 mL R1, 4 x 25 mL R2
OSR6234 4 x 53 mL R1, 4 x 53 mL R2
OSR6634 4 x 173 mL R1, 4 x 173 mL R2

For *in vitro* diagnostic use only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Urea Nitrogen in human serum and urine on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Measurements of urea nitrogen are used in the diagnosis and treatment of certain renal and metabolic disorders.

Urea nitrogen makes up approximately 75% of the total nonprotein nitrogen (NPN) fraction of the blood. It is synthesized in the liver from ammonia produced as a result of deamination of proteins. Filtration of urea from the blood into the urine by the renal glomeruli is the chief means of eliminating surplus nitrogen from the body.

Blood Urea Nitrogen (BUN) levels are a measure of kidney function and also of prerenal and postrenal conditions. Prerenal causes of elevated BUN include cardiac decompensation, water depletion or increased protein catabolism. Among the renal causes of increased levels are acute glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, and tubular necrosis. Any type of obstruction of the urinary tract is a postrenal cause for elevated BUN levels.[1] Both urea and creatinine are cleared by the renal glomeruli, however, urea is subsequently partially reabsorbed by the renal tubules, while creatinine is not. Consequently, serum urea nitrogen and serum creatinine determinations are frequently performed together in the differential diagnosis of kidney function.

METHODOLOGY

This Urea Nitrogen procedure is based on an adaptation of the enzymatic method of Talke and Schubert.[2] In this method, urea is hydrolyzed enzymatically by urease to yield ammonia and carbon dioxide. The ammonia and α-oxoglutarate are converted to glutamate in a reaction catalyzed by L-glutamate dehydrogenase (GLDH). Simultaneously, a molar equivalent of reduced NADH is oxidized.[3,4,5] Two molecules of NADH are oxidized for each molecule of urea hydrolyzed. The rate of change in absorbance at 340 nm, due to the disappearance of NADH, is directly proportional to the BUN concentration in the sample.

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

$$NH4^+ + \alpha\text{-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Serum

If the analysis is delayed, the sample should be refrigerated or frozen. Stability has been reported to be 24 hrs at room temperature (15 - 25°C), several days at 2 - 8°C, and 2 - 3 months when frozen ≤ -20°C.[1]

Urine

Urine specimens should be maintained at 2 - 8°C until analysis. Urine specimens can be preserved by maintaining the pH at less than 4.[6]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum free from hemolysis is the recommended specimen. If plasma must be used, anticoagulants without ammonium ions such as K2/K3 EDTA and lithium or sodium heparin are recommended.

Timed 24 hour urine specimens are recommended. Random collections may be appropriate if the laboratory has established its own performance characteristics.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Urea Nitrogen Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Tris buffer	100 mmol/L
NADH	≥ 0.26 mmol/L
Tetra-Sodiumdiphosphate	10 mmol/L
EDTA	2.65 mmol/L
α-Oxoglutarate	≥ 9.8 mmol/L
Urease (Jack Bean)	≥ 17.76 KU/L
ADP	≥ 2.6 mmol/L
GLDH (Beef Liver)	≥ 0.16 KU/L

Also contains preservatives.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

UREA R1	EUH208	May produce an allergic reaction.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
UREA R2	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Sodium Pyrophosphate, Decahydrate 1 - 2%
		Tris(hydroxymethyl)– aminomethane 2 - 5%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat # DR0070)

Urine Calibrator (Cat # DR0090)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR6634 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The Urea Nitrogen Reagents are ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the AU480, AU680, DxC 500 and DxC 500i analyzers.

3. Opened reagents are stable for 14 days when stored in the refrigerated compartment of the AU5800 / DxC 700 AU analyzers.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity, precipitate, or change in color in the Urea Nitrogen reagent may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

The frequency of calibration is every 14 days on AU480, AU680, DxC 500i and DxC 500 AU. The frequency of calibration is every 7 days on the AU5800 and DxC 700 AU. Calibration of the Urea Nitrogen procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institutes of Standard and Technology (NIST) Standard Reference Material (SRM) 909b for serum specimens. For urine specimens use Urine Calibrator (Cat # DR0090).

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventive maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mg/dL, for conversion to SI units (mmol/L) the result is multiplied by 0.357.

REPORTING RESULTS

EXPECTED RESULTS

Serum[7]	6 - 20 mg/dL
Urine[6]	7 - 16 g/24 hours

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[8] show that the following substances interfere with this BUN procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin:	No significant interference up to 20 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 500 mg/dL Intralipid*

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[9]

Reliable estimations of Urea can only be achieved if steps are taken to avoid contamination from Ammonia. Reagents on the carousel which contain/liberate Ammonia may contaminate Urea Nitrogen OSR6x34 . Avoid the use of ammonia containing reagents together with Urea to mitigate against atmospheric ammonia transfer. Please be advised that atmospheric ammonia may also be released by the use of certain laboratory cleaning products. Please contact your local Beckman Coulter representative for further information.

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Urea Nitrogen procedure is linear from 2 to 130 mg/dL for serum determinations, and from 20 to 1,300 mg/dL for urine determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

SENSITIVITY

Typical change in absorbance per minute for 1 mg/dL of Urea Nitrogen is 2.5 mAbsorbance.

METHODS COMPARISON

Reference[10]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 700 AU
X Method	AU5800
Slope	1.004
Intercept	0.182
Correlation Coeff. (r)	1.000
No. of Samples (n)	132
Range (mg/dL)	4.9 - 121.1

Patient Urine samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 700 AU
X Method	AU5800
Slope	1.008
Intercept	2.8
Correlation Coeff. (r)	1.000
No. of Samples (n)	125
Range (mg/dL)	94 - 1098

PRECISION

Reference[10]

Estimates of precision, based on CLSI recommendations[11], are consistent with typical performance. The within run precision for serum samples is less than 3% CV and total precision is less than 5% CV. Assays of control sera were performed and this data reduced following CLSI guidelines above.

Serum

N = 80	**Within-run**		**Total**	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
15.86	0.16	1.0	0.4	2.5
25.95	0.26	1.0	0.63	2.4
39.55	0.33	0.8	0.95	2.4
92.53	0.80	0.9	1.97	2.1
102.78	0.96	0.9	2.35	2.3

Urine

N = 80	Within-run		Total	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
119.16	0.83	0.7	1.60	1.3
396.27	2.37	0.6	3.99	1.0
1173.37	6.44	0.5	12.37	1.1

ADDITIONAL INFORMATION

DxC 700 AU analyzers require that each reagent application has a standard format of abbreviated Test Name. This Test Name is required to allow automated loading of the calibrator information for each application. Refer to the table below for the Test Name assigned to each application for this assay.

Test Name	Description
BUN1U	Urea (Serum)
BUN1U	Urea (Urine)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

Serum: † Beckman Coulter System Calibrator Cat No.: DR0070

Urine: † Beckman Coulter System Calibrator Cat No.: DR0090

* Values set for working in mg/dL. To work in SI units (mmol/L) divide by 2.8.

REVISION HISTORY

Add DxC 500i instrument to IFU

Preceding version revision history

Updated Specimen Section

Updated REAGENTS section

Updated REPORTING RESULTS section Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

REFERENCES

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