UREA NITROGEN

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 165 mL**	**R1**
	4 x 165 mL	**R2**

Intended Use
System reagent for the quantitative determination of Urea Nitrogen in human serum and urine on Beckman Coulter AU analyzers.
*Urea Nitrogen (Bun) reagent OSR6634 for use on the AU2700/5400 system only.

Summary
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.

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

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

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
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.

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

Preparation of Reagents
For OSR6134 and OSR6234, the Urea Nitrogen Reagents are ready for use. No preparation is required. For OSR6634, insert the pipe supplied into the 180 mL reagent vial before use on the analyzer. Care must be taken when handling the pipe to avoid contamination. The pipe is for single use only. Do not remove the large cap.

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 analyzer.

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.

Urea Nitrogen

Specimen Collection And Preparation

Serum free from hemolysis is the recommended specimen. If plasma must be used, anticoagulants without ammonium ions such as EDTA and lithium or sodium heparin are recommended. Timed, 24-hour collection is recommended for urine specimens which should be prediluted 1/10 with water before analysis on AU600 in standard Measure Mode.

Sample 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]

Interfering Substances
Results of studies[7] 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. For further information on interfering substances, refer to Young[8] for a compilation of reported interferences with this test.

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Urea Nitrogen Reagent
Pipe (one per each 180mL vial)

Materials Required But Not Provided
Chemistry Calibrator (Cat # DR0070)
Urine Calibrator (Cat # DR0090)

Stability Of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is every 14 days. 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 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 User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.
Appropriate qualified urine controls should be established and utilized during urine analysis.

Results
Automatically printed out for each sample in mg/dL at 37°C. For SI units (mmol urea/L) the result must be multiplied by 0.357.

Dynamic 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.

Expected Values

Serum:[9] 7 - 25 mg/dL
Urine:[6] 7 - 16 g/24 hours
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Urea Nitrogen Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[11]
Estimates of precision, based on CLSI recommendations[10], 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 = 100	Within run		Total	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
16.2	0.4	2.4	0.4	2.5
54.4	0.5	0.9	0.7	1.3

Urine

N = 100	Within run		Total	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
444	2.4	0.5	6.4	1.4
787	4.1	0.5	25.9	3.3

Method Comparison[11]

Serum

Patient samples were used to compare this Urea Nitrogen reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640/640[e]
X Method	AU600
Slope	1.000
Intercept	0.00
Correlation Coeff. (r)	0.999
No. of Samples (n)	184
Range (mg/dL)	4.0-96.0

Urine

Urine samples were used to compare this Urea Nitrogen reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.985
Intercept	+ 4.7
Correlation Coeff. (r)	0.999
No. of Samples (n)	137
Range (mg/dL)	90-1482

Sensitivity:

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

References

1. Tietz, N.W. (ed), Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders, 676, 1987.
2. Talke, H. and Schubert, G.E., Klinische Wochenschrift, 43: 174 1965.
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4. Roch-Ramel, F., Anal Biochem, 21: 372, 1967.
5. Reichelt, K.L., Kvamme, E. and Tveir, B., Scand J Clin Lab Invest, 16: 433, 1964.
6. Kaplan, L.A. and Pesce, A.J., Clinical Chemistry Theory, analysis and correlation, 3rd edition, C.V. Mosby, 1996.
7. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-A, 2002.
8. Young, D.S., Effect of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
9. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
10. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices EP5-A,1999.
11. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA

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