UREA NITROGEN (BUN) STAT

OSR6141	**4 x 25 mL**	**R1**	**Component 1**
	4 x 25 mL	**R1-2**	**Component 2**
OSR6541	**4 x 50 mL**	**R1**	**Component 1**
	4 x 50 mL	**R1-2**	**Component 2**

Intended Use
System reagent for the quantitative determination of Urea Nitrogen in human serum on Beckman Coulter AU analyzers.
Urea Nitrogen (BUN) reagent OSR6541 for use on the AU2700/5400/680 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.

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

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

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
To prepare the working STAT reagent, slowly add the contents of the bottle marked R1-2 (Component 2) to the bottle marked R1 (Component 1). Mix gently by inversion and place on board the instrument.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. The working STAT reagent is stable for 14 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.

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.

Urea Nitrogen (BUN) STAT

Sample Storage and Stability
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]

Interfering Substances
Results of studies[6] show that the following substances interfere with this Urea Nitrogen procedure.

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

Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Hemolysis: No significant interference up to 500 mg/dL Hemolysate
Lipemia: No significant interference up to 500 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

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[7] 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

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

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 7 days. Calibration of this 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.
Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed and 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.

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 140 mg/dL. 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:[8] 7 – 25 mg/dL

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[10]
Estimates of precision, based on CLSI recommendations[9], 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.

N = 100	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
16.0	0.1	0.9	0.3	1.6
54.4	0.5	0.9	0.5	1.0

Method Comparison[10]
Patient 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	0.988
Intercept	+0.3
Correlation Coeff. (r)	0.999
No. of Samples (n)	183
Range (mg/dL)	2.0-96.0

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.
3. Manoukian, E. and Fawaz, G.Z., Klin Chem Klin Biochem, 7: 32, 1969.
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. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-P, 1986.
7. Young, D.S., Effect of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
8. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
9. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices EP5-TS, 1992.
10. Data is on file for specific AU analyzers.

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

CE