POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Urea Nitrogen (BUN) Reagent Set

Intended Use
For the quantitative determination of urea nitrogen in serum.

Method History
Urea has been determined by the direct method[1] where urea condenses with diacetyl to form a chromagen and an indirect method where ammonia is measured as a product of Urease action on urea.[2] The liberated ammonia has been measured using Nessler's reagent[3] and by the Berthelot reaction.[4] Talke and Schubert introduced a totally enzymatic procedure in 1965 utilizing Urease and Glutamate Dehydrogenase.[5] The present procedure is based on a modification of their method.

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

$$NH_3 + \alpha\text{-Ketoglutarate} + NADH + H^+ \xrightarrow{\text{GD}} \text{L-glutamate} + NAD^+ + H_20$$

Urea is hydrolyzed by Urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with a-Ketoglutarate in the presence of NADH to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction resulting in a decrease in absorbance that is directly proportional to the urea nitrogen concentration in the sample.

Reagents
(Concentrations refer to reconstituted reagent)
NADH 0.3mM, Urease 1,500 U/L, Glutamate Dehydrogenase > 1500 U/L, α-Ketoglutarate 4.0 mM, Buffer pH 8.2 ± 0.1, Activators and non-reactive stabilizers.

Reagent Preparation
Reconstitute reagent with the volume of distilled water stated on the vial label, swirl to dissolve.

Reagent Storage
1. Store reagent at 2-8°C.
2. Store reconstituted reagent at 2-8°C.
3. Reconstituted reagent is stable 2 days at 18-25°C and 30 days at 2-8°C.

Reagent Deterioration
Do not use if:
1. Moisture has penetrated the vial and caking has occurred.
2. The reconstituted reagent has a reagent blank absorbance less than 1.0 at 340 nm.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion of reagent as toxicity has not yet been determined.

Specimen Collection and Storage
1. Serum is recommended.
2. Plasma containing anticoagulants should not be used.
3. All material coming in contact with the sample must be free of ammonia and heavy metals.[6]
4. Urea in serum is reported stable for seventy-two hours refrigerated at 2-8°C. Unrefrigerated sera should be used within eight hours.

Interferences
1. Urease action is inhibited by fluoride.
2. Samples with abnormal ammonia levels give falsely elevated BUN results.
3. For a comprehensive review of drug interference see Young, et al.[7]

Materials Provided
Urea Nitrogen reagent.

Materials Required but not Provided
1. Accurate pipetting devices
2. Timer
3. Test tubes/rack
4. Spectrophotometer with a temperature controlled cuvette

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Reconstitute reagent according to instructions.
2. Zero spectrophotmeter with water at 340 nm.
3. Pipette 1.0ml of reagent into test tubes and allow reagent to come to room temperature.
4. Add 0.01ml (10ul) of sample to test tube and immediately place in the spectrophotometer.
5. After thirty seconds read and record the absorbance (A_1).
6. Sixty seconds after the first reading take another reading (A_2).
7. Determine the absorbance change between the two readings (A_1-A_2).
8. Repeat procedure for each sample.
9. See "Calculations" for determination of results.

Alternative Volumes
If the spectrophotmeter being used requires a final volume greater than 1.0ml for accurate reading, use 0.025ml (25ul) of sample to 3.0ml of reagent. Perform the test as described above.

Limitations
Samples with values above 80 mg/dl should be diluted with 0.9% saline 1:1, re-assayed and the results multiplied by two.

Calibration
Use an aqueous BUN standard (20 mg/dl) or an appropriate serum calibrator.

Calculations
(A_1-A_2) = Absorbance change between readings

$$\frac{(A_1 - A_2)\ \text{unknown}}{(A_1 - A_2)\ \text{standard}} \times \frac{\text{concentration}}{\text{of standard}} = \text{BUN (mg/dl)}$$

Example: If the unknown had an $A_1 = 1.5$ and $A_2 = 1.0$,
the standard $A_1 = 1.5$ and $A_2 = 0.9$ and
the concentration of the standard = 20 mg/dl then:

$$\frac{(1.5 - 1.0)}{(1.5 - 0.9)} = \frac{0.5}{0.6} \times 20 = 17\ \text{mg/dl}$$

NOTE: To obtain results in SI units multiply by 10 to convert dl to liters and divide by 28, the molecular weight of nitrogen.

Example: 17 mg/dl x 10/28 = 6.06 mmol/L.

To convert mg/dl Urea Nitrogen to mmol Urea/L, multiply the mg/dl Urea Nitrogen value by 0.357.

To convert mg/dl Urea Nitrogen to mg/dl Urea, multiply the mg/dl Urea Nitrogen value by 2.14.

Quality Control

Serum controls with known normal and abnormal values should be run routinely to monitor the validity of the reaction.

Expected Values

7-18 mg/dl[6]
It is strongly recommended that each laboratory establish its own reference range.

Performance

1. Linearity: 80 mg/dl
2. Comparison: A study performed using another enzymatic procedure yielded a correlation coefficient of 0.999 with a regression equation of y = 0.98 + 0.64.
3. Precision:

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
14	0.5	3.6	14	0.6	4.3
46	0.8	1.7	49	1.8	3.7

References

1. Fearon, W.R., Biochem J. 331:902 (1939).
2. Marshall, E.K., Jr., J. Biol. Chem. 15:487 (1913).
3. Gentzkow, C.J., J. Biol. Chem. 143:531 (1952).
4. Fawcett, J.K., Scott, J.E., J. Clin. Path. 13:156 (1960).
5. Talke, H., Schubert, G.E., Klin. Wschr. 43:174 (1965).
6. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia W.B. Saunders, p991 (1976).
7. Young, D.S., et al, Clin. Chem. 21:1D (1975).

Manufactured by Pointe Scientific, Inc.
5449 Research Drive Canton, MI 48188

"European Authorized Representative"
(O.E.A.R.C.) Av. De Tervueren 34 bte
44 B-1040 Brussels, Belgium

CE

Rev. 12/03 P803-B7550-01