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BUN REAGENT SET (UV-KINETIC METHOD)

UREA NITROGEN (BUN) REAGENT SET
For the determination of urea nitrogen in human serum.

INTRODUCTION
Urea is the major end product of protein nitrogen metabolism. It is synthesized in the liver from ammonia, which is produced by amino acid deamination. The determination of serum urea nitrogen is an important index of kidney function. Impaired renal function or increased tissue protein breakdown are associated with increased urea nitrogen levels, whereas liver damage or pregnancy are associated with decreased levels.[1]

In 1965 Talke and Schubert introduced a procedure utilizing urease and glutamate dehydrogenase (GD).[2] Tiffany et. al. later modified this system to a kinetic procedure that reduced the reaction time and allowed direct sample addition.[3] This formulation takes advantage of the kinetic method, providing a rapid assay for the quantitative determination of urea nitrogen.

PRINCIPLE
The enzymatic reaction sequence employed in the assay of BUN is as follows:

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{2-Oxoglutarate} + NADH + H^+ \xrightarrow{GD} \text{L-Glutamate} + NAD^+ + H_20$$

Urea in the sample is hydrolyzed by urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with 2-oxoglutarate, in the presence of GD and the coenzyme NADH, to produce L-glutamate. In this reaction 2 moles of NADH are oxidized to NAD for each mole of urea hydrolyzed. The resulting decrease in absorbance of NADH at 340 nm is proportional to the level of urea nitrogen in the sample.

REAGENT COMPOSITION
When reconstituted as directed, our reagent for BUN contains the following:
1. BUN Reagent: (Concentrations refer to the reconstituted reagent,) NADH 0.28 mM/L, Urease 3,000 U/L, Glutamate Dehydrogenase 15,000 U/L, 2-Oxoglutarate 4.0 mM/L, Buffer pH 7.8, Activators and non-reactive stabilizers.
2. Urea Nitrogen Standard (20 mg/dl): Urea.

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. The reagents contain sodium azide, which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.
3. Human serum specimens should be considered infectious and handled appropriately.

STORAGE AND STABILITY
Both the BUN reagent and Standard must be stored at 2 - 8° C prior to reconstitution. The reagent may be used until the expiration date indicated on the package label. After reconstitution the reagent is stable for two (2) days at room temperature (18 - 25° C) and for twenty-one (21) days when stored at 2 - 8° C. The reagent should be clear and colorless.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. Moisture has penetrated the vial and caking has occurred.
3. The reconstituted reagent has a reagent blank absorbance less than 1.0 at 340 nm (1cm L.P).

SPECIMEN COLLECTION
1. Test specimens should be serum free from hemolysis.
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.[4]
4. Urea in serum is reported stable for seventy-two hours refrigerated at 2-8 °C. Unrefrigerated serum should be used within eight hours.

INTERFERING SUBSTANCES
Anticoagulants such as fluoride, citrate and EDTA may inhibit urease and should be avoided. Ammonium ions present in water or other substances may falsely elevate urea values. Young et al. give a comprehensive review of drug interferences.[5]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipettes to accurately measure required volumes.
2. Test tubes/rack.
3. Timer.
4. Distilled or deionized water where indicated.
5. Spectrophotometer with a temperature controlled cuvette.

GENERAL INSTRUCTIONS
The reagent for BUN is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Refer to appropriate application manual available from Teco.

PROCEDURE (MANUAL)
1. Reconstitute reagent according to instructions.
2. Zero spectrophotometer with water at 340 nm.
3. Pipette 1.0 ml of BUN reagent into test tubes and pre-heat to 37 °C.
4. To one cuvette at a time add 0.01ml (10 µl) of sample (standard or serum).
5. After thirty seconds measure and record the absorbance (A1).
6. After an additional sixty seconds take a second absorbance reading (A2).
7. Determine the ΔA between the two readings (A1 - A2).
8. Repeat procedure for each sample.

* TC - MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

NOTE:

- *For higher linearity, read only for 30 seconds instead of 60 seconds as called for in the procedure.*
- *If the spectrophotometer being used requires a final volume greater than l.0 ml for accurate reading, use 0.025 m1 (25µ1) of sample to 3.0 ml of reagent Perform the test as described above.*

PROCEDURAL LIMITATIONS

The reagent is linear to 80 mg/dl urea nitrogen. Samples with values above 80 mg/dl should be diluted 1:1 with 0.9% saline, reassayed and the results multiplied by 2.

CALCULATIONS

$(A_1 - A_2)$ = Absorbance change between

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

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

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

SI UNITS:

$$\text{mg/dl} \times \frac{10}{28} = \text{mg/dl} \times 0.357$$

Where 10 = Conversion of dl to liter
28 = molecular weight of nitrogen

Example: If 17 mg/dl is the result then 17 x 0.357 = 6.06 mMol/L

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established BUN values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

7-18 mg/dl[4]

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

1. Linearity: 80 mg/dl
2. Comparison: A comparison using enzymatic procedure yielded a correlation coefficient of 0.96 with a regression equation of y - 0.95x + 3.67
3. Precision studies:

	Within Run	
Mean (mg/dl)	S.D.	C.V.
12	0.5	4.6%
43	0.4	1.0%

	Run to Run	
Mean (mg/dl)	S.D.	C.V.
12	0.5	4.6%
43	1.6	3.8%

REFERENCES

1. Henry, J.B., Todd, Sanford, Davidsohn: *Clinical Diagnosis and Management by Laboratory Methods,* 16th ed., W.B. Saunders and Co., Philadelphia, PA. p260 (1974).
2. Talke, H. Schubert, G.E.: *Klin. Wchenschr* 43:174 (1965).
3. Tiffany, T. 0., Jansen, J.M., Burtis, C.A., Overton, J.B., and Scott. C.D.: *Clin. Chem*. 18:829 (1972).
4. Tietz, N.W.: *Fundamentals of Clinical Chemistry*, Philadelphia, W.B. Saunders, and Co., Philadelphia, PA. p991 (1976).
5. Young, D.S., et. al: "Effects of Drugs on Clinical Lab. Tests." *Clin. Chem*., 18 ID-432D (1972).

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