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UREA NITROGEN (BUN) REAGENT (COLORIMETRIC METHOD)

Catalog Number: BQ 009B-CR

INTENDED USE

For the direct colorimetric determination of Urea Nitrogen (BUN) in human serum or plasma.

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 determination. 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]

PRINCIPLE

The Bertelot reaction has long been used for the measurement of urea and ammonia.[2] The present method is a modified Berthelot.

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

NH_3 + Salicylate + Hypochlorite → 2, 2-Dicarboxyindophenol

The BUN colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonium by the use of urease. Ammonium ion then reacts with a mixture of salicylate, sodium nitroprusside, and hypochlorite to yield a blue-green chromophore. The intensity of the color formed is proportional to the urea concentration in the sample.

REAGENT COMPOSITION

When reconstituted as directed, the reagent for BUN contains the following:

1. BUN Enzyme Reagent: After reconstitution with distilled water, BUN enzyme reagent contains the following minimum concentrations of reactants: Urease 10,000U/L, Sodium Salicylate 6.0mmol/L, Sodium Nitroprusside 3.2 mmol/L, stabilizers, and a buffer.
2. BUN Color Developer: BUN Color Developer contains Sodium Hypochlorite, 6 mmol/L and Sodium Hydroxide, 130 mmol/L.
3. Urea Nitrogen Standard (20 mg/dl): A stabilized solution of urea equivalent to 20 mg of Urea Nitrogen/l00ml.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. BUN Enzyme Reagent and BUN Color Developer contain poisons and should not be pipetted by mouth.
3. BUN Color Developer may be irritating to the skin. Avoid contact.
4. Serum specimens should be considered infectious and handled appropriately.

STORAGE AND STABILITY

All the BUN Colorimetric Reagents and Standard must be stored at refrigerated temperature (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 ten (10) days at room temperature (18 – 30 °C) and for ninety days (90) when stored at refrigerated temperature. The reagent should be clear and slightly yellow.

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.

SPECIMEN COLLECTION

1. Test specimens should be human serum and free from hemolysis.
2. Plasma may be substituted provided the anticoagulant used is free of ammonium salt.
3. All material coming in contact with the sample must be free of ammonia and heavy metal.[3]
4. Urea in serum is reported stable for seventy-two (72) hours refrigerated at 2 – 8 °C. Un-refrigerated serums should be used within eight (8) 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[4].

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 Colorimetric determination is intended for use as a manual procedure on a suitable spectrophotometer.

PROCEDURE (MANUAL)

1. Reconstitute BUN Enzyme Reagent according to the instructions.
2. Pipette 1.5 ml of BUN Enzyme Reagent into labeled test tubes. Allow to equilibrate to room temperature.
3. Add 0.010 ml (10 µl) of each sample to its respective tube. Mix gently. Use deionized water as the sample for the Reagent Blank.
4. Incubate all tubes for five minutes (5) at 37 °C for ten minutes (10) at room temperature (18 – 30 °C)
5. Add 1.5ml BUN Color Developer. Mix gently.
6. Incubate for five minutes (5) at 37 °C or ten minutes (10) at room temperature (18 – 30 °C).
7. Zero spectrophotometer with the Reagent Blank at 630nm. (Wavelength range: 580 – 630nm)
8. Read and record the absorbance of samples of all tubes.

* MULTI PURPOSE CALIBRATOR MAY BE USE TO REPLACE STANDARD.

PROCEDURAL LIMITATIONS

The reagent is linear to 100mg/dl Urea Nitrogen. Samples with values above 100mg/dl should be diluted 1:1 with 0.9% saline, re-assayed, and the results multiplied by two (2).

CALCULATIONS

Use the equation below to calculate BUN concentrations of the samples.

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of Std.} = \text{Urea Nitrogen (mg/dl)}$$

Example:

Absorbance of sample = 0.185
Absorbance of standard = 0.303

$$\frac{0.185}{0.303} \times 20 \text{ mg/dl} = 12.2 \text{ mg/dl}$$

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 reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES[5]

7-23 mg/dl

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

PERFORMANCE CHARACTERISTICS

1. Linearity: 100 mg/dl
2. Comparison: A comparison using enzymatic procedure yielded a correlation coefficient of 0.97 with a regression equation of: $Y = 0.99x + 0.02$.
3. Precision studies:

Within Run

Mean (mg/dl)	S.D.	C.V.
16.3	0.083	5.1%
50.7	1.62	3.2%

Run to Run

Mean (mg/dl)	S.D.	C.V.
16.4	0.7	4.3%
51.4	0.96	1.8%

REFERENCES

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3. Tietz, N.W.: *Fundamentals of Clinical Chemistry* Philadelphia, W.B. Saunders, and Co., Philadelphia, PA. p991 (1976).
4. Young, D.S. et.al: "Effects of Drugs on Clinical Lab. Tests." Clin. Chem., 18 ID-432D (1972).
5. Wildmann, F.K.: *Coodales Clinical Interpretation Laboratory Tests.* , F.A. Davis Co., Philadelphia, (1969).