

BUN Color

TEST PROCEDURE FOR SPECTROPHOTOMETER

The following is a general procedure for use on a manual spectrophotometer.

Procedure Conditions

Wavelength	 540 nm
Temperature	37° C
Mode	Endpoint
Sample to Reagent Ratio	1:70

TEST PROCEDURE (1)

- A. Label reagent tubes, as "BLANK", "STANDARD" and "PATIENT".
- B. Add 1 ml of BUN reagent to each reagent tube.
- C. Add 0.5 ml of color developer solution to each reagent tube. Mix well.
- D. Add 50 µl of BUN standard to the "STANDARD" tube. Mix well.
- E. Add 50 µl of patient serum to the "PATIENT" tube. Mix well.
- F. Incubate reagent tubes at 37° C for exactly 5 minutes.
- G. Add 2 ml of diluent to each reagent tube. Mix well.
- H. Wipe the reagent tubes clean with a lint-free tissue.
- I. Place the "BLANK" reagent tube in the test well and adjust the photometer to zero absorbance.
- J. Place the "STANDARD" and "PATIENT" tubes in the test well and record the absorbance of the "STANDARD" and "PATIENT" samples.

Calculation Patient BUN concentration =

$$\frac{\text{absorbance of "PATIENT" sample}}{\text{absorbance of "STANDARD" sample}} \times \text{X value of standard}$$

EXPECTED VALUES Normal range: 8 - 23 mg/dl

- NOTE:
1. (a) Bilirubin has no effect if present in concentrations of less than 20 mg/dl and hemoglobin has no effect if present in concentration of less than 150 mg/dl.
 - (b) Sulfanilamide and its derivative produce false elevations.

INTENDED USE

Quantitative determination of Blood Urea Nitrogen (BUN) in serum.

SUMMARY AND EXPLANATION

The two commonly used methods for measuring urea nitrogen are the diacetyl

reaction (1) and urease phenol and hypohalite reaction (2). In 1975 Jung and Biggs (3) developed a method to directly measure urea nitrogen. This method does not require elevated temperatures for color development, uses nonvolatile and stable reagents and has specificity comparable to that of the diacetyl reaction. The Biotron Diagnostics method is the modification of this new colorimetric reaction for end point measurement of urea nitrogen.

TEST PRINCIPLE

Urea reacts with o-phthalaldehyde to form isoindoline which reacts with Naphthylethylenediamine to form a chromophore whose color intensity is directly proportional to the concentration of urea nitrogen.

MATERIALS PROVIDED

BUN reagent	132 ml
Color developer	66 ml
Diluent	264 ml
Standard/Control/Calibrator	

REAGENTS

For In Vitro Diagnostic use.

BUN Reagent - contains 0.6% o-phthalaldehyde, 9.5% of concentrated sulfuric acid and a surfactant.

Color developer - contains 0.5% naphthylethylenediamine, 6% of boric acid and a surfactant.

Diluent - contains 0.1% of concentrated sulfuric acid.

CAUTION! Do not take these reagents internally or allow them to come in contact with the body.

STORAGE

Store reagents at room temperature (18-26° C). All reagents are stable till the expiration date stated on the label when stored at room temperature.

ADDITIONAL MATERIALS REQUIRED

Spectrophotometer, colorimeter or blood analyzer
Pipetting device
Reagent Tubes or Cuvettes
37° C heat source

SAMPLE PREPARATION

Freshly drawn, fasting serum is the specimen for Biotron Diagnostics BUN test.

EXPECTED VALUES (4)

Normal BUN range is 8-23 mg/dl.

The above range is intended as a guide. Each laboratory should establish its own normal range.

PERFORMANCE (5)

1. Precision - The precision study was done by
 - (a) repetitive assay (N=20) of normal serum specimen. This assay yielded a mean of 12.4 mg/dl, a standard deviation of 0.51 mg/dl and a coefficient of variation of 4.1%.
 - (b) repetitive assay (N=20) of abnormal serum specimen. This assay yielded a mean of 55.2 mg/dl, a standard deviation of 1.5 mg/dl and a coefficient of variation of 2.7%.
 - (c) 8 day reproducibility study. A pool serum specimen with a mean of 15.7 mg/dl yielded a standard deviation of 0.58 mg/dl and a coefficient of variation of 3.7%.
2. Accuracy - The accuracy study was done by
 - (a) running 23 serum specimens on SMA 12/60 (registered trademark of Technicon Corporation) procedure and Biotron Diagnostics method on Gilford Stasar III (registered trademark of Gilford Instrument). The study yielded a regression equation of $\text{Biotron} = 1.036 \times \text{reference method} - 0.85$ and a correlation of 0.98.
 - (b) adding known BUN aqueous standards of varying concentration with Biotron Diagnostics method. Recovery was in the range of 97.5% to 101%.

LIMITATIONS OF THE PROCEDURE

This procedure is linear through 70 mg/dl beyond which the specimen should be diluted 1:2 with deionized water. Reassay the specimen and multiply the result by 2.

QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used. Daily quality control must fall within 2 standard deviations of the established value. If correlation is not obtained and repetition of the assay excludes errors in technique, the following steps should be taken:

1. Calibrate the instrument according to manufacturer's instructions.

2. Check the expiration date of the reagent package.
3. Check the cleanliness of the reagent tube.
4. Contact Biotron Diagnostics Technical Services Department.

REFERENCE

1. Henry, R.J., Clinical Chemistry, Principles and Techniques, Harper and Row, N.Y., N.Y. 1967, p.266.
2. Marsh, W.H., Fingerhut, B. and Millard, H. "Clinical Chemistry" 11:624, 1965.
3. Jung, D. et al "Clin Chem" 21:1136, 1975.
4. Tietz, N.W. "Fundamentals of Clinical Chemistry" W.B. Saunders, Philadelphia, 1970.
5. Kin Diagnostics Laboratory Data, Indianapolis, IN, 1981.