

# Blood Urea Nitrogen (Liquid)

## 1.0 INTENDED USE

This reagent is intended for the quantitative determination of urea nitrogen (BUN) in serum.

## 2.0 BACKGROUND

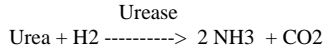
### 2.1 METHOD AND HISTORY

Urea has been determined by the direct method (10.2) 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. (10.3)

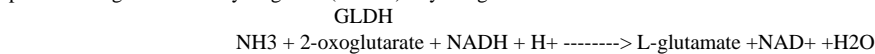
The liberated ammonia has been measured using Nessler's reagent (10.4) and by the Berthelot reaction. (10.5) Talke and Schubert introduced a totally enzymatic procedure in 1965 initializing Urease and Glutamate Dehydrogenase. (10.6) The present procedure is based on a modification of their method.

### 2.2 TEST PRINCIPLE

In the Talke and Schubert procedure urea is first hydrolyzed by urease to give ammonia and carbon dioxide as in the equation:



In the second step of the process the ammonia produced in the first reaction reacts with 2-oxoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NAD.



Urea is hydrolyzed by Urease to produce ammonia and water. The liberated ammonia reacts with  $\alpha$ -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.

### 2.3 CLINICAL SIGNIFICANCE (10.1)

Determination of urea nitrogen in serum is widely used as a screening test for renal function. When used in conjunction with the determination of creatinine in serum it is helpful in the differential diagnosis of the three types of azotemia; prerenal, renal and post-renal.

## 3.0 SPECIMEN COLLECTION AND HANDLING

### 3.1 PATIENT PREPARATION

No special patient preparation is required.

### 3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. The serum should be promptly separated from the clot. Fluoride is a known inhibitor of urease. Therefore anticoagulants containing fluoride should be avoided. (10.8)

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25  $\mu$ l. Call Biotron's technical service department at 1-800-262-8655 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

### 3.3 SPECIMEN STORAGE

Samples may be stored refrigerated (2-8°C) for 3-5 days or frozen (-20°C) for several months.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

## 4.0 MATERIALS (2 x 125 ml)

Reagents necessary for the determination of BUN are included in the kit.

### 4.1 REAGENT

BUN working reagent contains:

|                                  |             |
|----------------------------------|-------------|
| alpha-ketoglutarate              | 4.0 mM      |
| ADP                              | 2.0 mM      |
| NADH                             | 0.28 mM     |
| urease                           | >15,000 U/L |
| glutamate dehydrogenase (bovine) | >1667 U/L   |
| sodium azide                     | 0.2%        |
| buffer pH 7.6 +/- 0.1            |             |

#### 4.1.1 Standard/Control/Calibrator

### 4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water.

### 4.3 REAGENT PREPARATION

The working reagent is ready to use as is.

### 4.4 REAGENT STORAGE AND STABILITY

Store reagents in refrigerator. Protect from light and freezing. Unopened reagents are stable at 2-8°C (refrigerated) until the expiration date stated on the labels. The working reagent is stable for 14 days at 2-8°C and 3 days at 18-26°C.

### 4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 340 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 340 nm.

4.5.3 Test tubes and pipettes.

4.5.4 Timer.

4.5.5 Constant temperature source which can be adjusted to 37°C.

4.5.6 Normal and abnormal controls for quality control.

**5.0 TEST PROCEDURE**

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

Application procedures for use on automated analyzers are available. Contact Biotron's Technical Service Department for specific information.

**5.1 PROCEDURE CONDITIONS**

|                         |            |
|-------------------------|------------|
| Wavelength              | 340 nm     |
| Temperature             | 37° C      |
| Pathlength              | 1.0 cm     |
| Mode                    | endpoint   |
| Lag time                | 30 sec     |
| Read time               | 60 sec     |
| Sample to reagent ratio | 1:100      |
| Reaction direction      | decreasing |

**5.2 INSTRUMENT**

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

**5.3 CALIBRATION**

Use Biotron's serum based calibrator. The BUN assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

**5.4 PROCEDURE**

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

5.4.1 Prepare the required volume of working reagent (see 4.3 Reagent Preparation Section.)

5.4.2 Adjust the absorbance reading at 340 nm on the spectrophotometer to 0.000 using distilled water as the blank.

5.4.3 Pipette 1.0 ml of working reagent into test tubes. Determine the absorbance of the working reagent at 340 nm. This should be at least 1.0.

5.4.4 Into separate test tubes pipette 10 µl of calibrator or serum to be assayed.

5.4.5 Incubate at 37° C for 30 seconds. Record the absorbance reading A1.

5.4.6 Incubate for another 1 minute at 37° C. Record the absorbance (A2).

5.4.7 Record the change in absorbance  $\Delta A$  (A2-A1).

## 5.5 CALCULATION AND RESULTS

$$\text{Urea} = \frac{\Delta A}{\Delta A_s} \times \text{concentration of calibrator}$$

$\Delta A$  = change in absorbance of sample  
 $\Delta A_s$  = change in absorbance of the calibrator  
Example:

$$\text{Urea} = \frac{0.165}{0.372} \times 30 \text{ mg/dl} = 13.3 \text{ mg/dl}$$

with  $\Delta A = 0.165$ ,  $\Delta A_s = 0.372$ , concentration of calibrator = 30 mg/dl

## 6.0 INTERPRETATION OF RESULTS

### 6.1 EXPECTED VALUES

The range of expected values is: 8-26 mg/dl  
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

### 6.2 MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

### 6.3 LIMITATIONS OF PROCEDURE

Urease is specific for urea, however ammonia contamination will seriously affect the results obtained using the system. Analysis should not be performed in close proximity to a urinalysis laboratory or in a laboratory using cleaning supplies containing ammonia.

A summary of the influence of drugs of clinical laboratory tests may be found by consulting Young D.S., Et. Al.(10.7). Severely icteric, hemolytic, or lipemic samples require the use of a sample blank which may be prepared using 10  $\mu$ l of sample and 3 ml of deionized water.

## 7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

## 8.0 CALIBRATION PROCEDURES

The BUN assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by QC data.

## 9.0 PERFORMANCE CHARACTERISTICS

### 9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

| Within-Run   |            |        |
|--------------|------------|--------|
| Mean (mg/dl) | SD (mg/dl) | CV (%) |
| 10.1         | $\pm 0.3$  | 3.1    |
| 68.4         | $\pm 0.6$  | 1.4    |
| 144.3        | $\pm 1.9$  | 1.3    |
| Between-Run  |            |        |
| Mean (mg/dl) | SD (mg/dl) | CV (%) |
| 10.3         | $\pm 0.5$  | 4.6    |
| 69.3         | $\pm 0.9$  | 1.3    |
| 145.1        | $\pm 2.3$  | 1.6    |

### 9.2 CORRELATION

A correlation study was done by running 107 specimens, ranging from 5 to 139 mg/dl, with a similar comparative method.

| Number of Samples | Regression Equation | Correlation Coefficient |
|-------------------|---------------------|-------------------------|
| 107               | $y = 1.05x - 0.2$   | 0.999                   |

### 9.3 LINEARITY

This procedure is linear through 150 mg/dl beyond which the specimen should be diluted 1 to 1 with deionized water. Reassay the specimen and multiply the results by 2.

## 10.0 REFERENCES

- 10.1 Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, (1976).
- 10.2 Fearon, W.R., Biochem J. 331:902(1939).
- 10.3 Marshall, E.K., Jr., J. Biol. Chem. 15:487(1913).
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- 10.5 Fawcett, J.K., Scott, J.E., J. Clin. Path. 13:156(1960).
- 10.6 Talke, H., Schubert, G.E., Klin. Wschr. 43:174(1965).
- 10.7 Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia W.B. Saunders, p.991(1976).
- 10.8 NCCLS document Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue, 2nd Ed.(1991).

10.9 Young D.S., et al, Clin. Chem. 21:1D(1975).

10.10 NCCLS document Evaluation of Precision Performance of Clinical Chemistry Devices:, 2nd Ed.(1992).