

BUN (Kinetic)

1.0 INTENDED USE

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

2.0 BACKGROUND

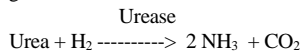
2.1 METHOD AND HISTORY

The measurement of urea nitrogen (BUN) has traditionally been performed by either condensation with diacetyl monoxime or by conversion of urea by urease to ammonia. Fearon (10.1) first proposed the diacetyl monoxime method in 1939. This colorimetric method has limitations such as poor specificity and color instability. The use of urease in BUN determinations was introduced by Marshall (10.2) who measured the liberated ammonia by titration with acid. Ammonia produced by the urease action has also been measured by Nesslerization techniques (10.3,10.4) and by the Berthelot reaction (10.5). These colorimetric methods lack specificity, require long incubation periods and require high temperatures.

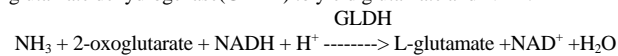
This procedure is based on Talke and Schubert (10.6), who described the first totally enzymatic procedure for measuring urea. In this procedure, the urease reaction is coupled to the concurrent amination of 2-oxoglutarate and the oxidation of NADH by glutamate dehydrogenase.

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.



The oxidation of NADH causes a decrease in absorbance at 340 nm which is proportional to the concentration of urea in the sample.

2.3 CLINICAL SIGNIFICANCE (10.8)

Serum levels of urea will increase in renal failure as the glomerular filtration rate decreases.

There is a small but significant postprandial increase in serum urea caused by the catabolism of ingested proteins.

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 µl. Call Biotron's technical service department at 1-800-595 8766 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

(10 X 10 ml)
(6 X 50 ml)
(6 X 100 ml)
(6 X 500 ml)

Reagents necessary for the determination of urea nitrogen are included in the kit.

4.1 REAGENT

Urea nitrogen reagent contains, after reconstitution with deionized water:

2-oxoglutarate	4.0 mM
ADP	2.2 mM
NADH	0.37 mM
urease	≥10,000 U/L
glutamate dehydrogenase	≥5,000 U/L
buffer	

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.

4.3 REAGENT PREPARATION

4.3.1

Reconstitute each urea reagent vial with 10 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.2

Reconstitute each urea reagent vial with 50 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.3

Reconstitute each urea reagent vial with 100 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.4

Reconstitute each urea reagent vial with 500 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

Store reagents in refrigerator. Protect from light and freezing. All reagents included in the kit are stable at 2-8° C (refrigerated) until the expiration date stated on the labels. The reconstituted reagent is stable for 5 days when stored at 2°-8° 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 capable of holding 4 ml.

4.5.4

Pipettes capable of delivering 3 ml and 10 µl.

4.5.5

Deionized or distilled water for preparing the reagent blank.

4.5.6

Timer for a 5 minute or 10 minute incubation.

4.5.7

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

4.5.8

Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

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

5.1 PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37° C, or 18-26° C
Pathlength	1.0 cm
Mode	endpoint
Reaction time	5 min at 37° C 10 min at 18-26° C
Sample volume	10 µl
Reagent volume	3ml
Total volume	3.01 ml
Sample to reagent ratio	1/300

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

5.4 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 Determine the absorbance (Ar) of the reconstituted reagent at 340 nm. (Ar) should be at least 1.6.
- 5.4.4 Into separate test tubes pipette 10 µl of calibrator or serum to be assayed.
- 5.4.5 Add 3.0 ml of reagent and mix.
- 5.4.6 Incubate for 5 minutes at 37° C in a heat block or 10 minutes at 18-26° C (room temperature) and determine the absorbance of the calibrator (As) and of each serum (A) at 340 nm using distilled water as the blank.

5.5 PROCEDURE NOTE

The final reaction mixture is stable for 5 minutes.

5.6 CALCULATION AND RESULTS

$$Ar - A$$

Urea = ----- X concentration of calibrator

$$Ar - As$$

Ar = initial absorbance of the reagent

A = absorbance of the unknown

As = absorbance of the calibrator

Example:

$$1.950 - 1.785$$

Urea = ----- X 30 mg/dl = 13.3 mg/dl

$$1.950 - 1.578$$

with Ar = 1.950, A = 1.785, As = 1.578, concentration of calibrator = 30 mg/dl

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.8)

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 (10.9)

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 µl 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 quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

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

Within-Run

In this study, 30 replicates of 2 control sera were run.

Mean (mg/dl)	SD (mg/dl)	CV (%)
13.7	± 1.16	8.5
46.1	± 2.06	4.5

Between-Run

In this study, 5 replicates of 2 control sera were run each day for 10 days.

Mean (mg/dl)	SD (mg/dl)	CV (%)
15.0	± 0.63	4.2
46.3	± 1.0	2.2

9.2 CORRELATION

A correlation study was done by running 44 specimens, ranging from 10 to 70 mg/dl, with a similar comparative method (Gilford) and this method on Gilford Stasar III (registered trademark of Gilford Instruments).

Number of Samples	Regression Equation y=Biotron, x=Comparative	Correlation Coefficient
44	y = .952 x + 1.63	0.991

9.3 LINEARITY

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

9.4 RECOVERY

The recovery study was done by adding known urea standards of varying concentrations to 3 pools of human serum to increase the urea concentrations to 20.1, 43.2 and 67.2 mg/dl. With this method, average recovery is 96%.

10.0 REFERENCES

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- 10.5 Fawcett, J.K. and Scott, J.E., J. Clin. Pathol. 13,156 (1960).
- 10.6 Talke, H., Schubert, G.E., Enzymatische Harnstoffbestimmung in BLUT and Serum in Optischcen Test NACH Warburg, Klin. Wochschr 43,174 (1965).
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- 10.9 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704

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