MEDICA

IVD CE

REF 10202-4 4 x 39 mL

BLOOD UREA NITROGEN (BUN)

Wedges each contain a usable volume of 39 mL of reagent.

INTENDED USE

The EasyRA BUN reagent is intended for the quantitative measurement of Blood Urea Nitrogen (BUN) in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA "EasyRA® Clinical Chemistry Analyzer." Blood Urea Nitrogen measurements are used in the diagnosis and treatment of certain renal and metabolic diseases.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Urea, the major end product of amino acid degradation, is primarily excreted by the kidneys. Serum urea is used as a test of renal function. In conjunction with serum creatinine determination, urea measurement can aid in the differential diagnosis of azotemia.[1]

PRINCIPLE OF THE PROCEDURE

The BUN reagent is based on the Talke and Schubert method.[2]

In these two coupled enzymatic reactions, Urea is first hydrolyzed by urease to give ammonia and carbon dioxide (I):

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

The ammonia produced in the first reaction reacts with 2-oxoglutarate and stabilized NADH analog[3] in the presence of glutamate dehydrogenase (GLDH) to form glutamate and NAD (II).

$$NH_3 + \text{2-oxoglutarate} + \xrightarrow{\text{GLDH}} \text{L-glutamate} + \text{NAD}$$

The decrease in the concentration of the reduced cofactor (NADH), monitored at 340 nm is proportional to the concentration of the Urea in the sample.

REAGENT

a-ketoglutarate	14 mM
Urease (botanical)	>50 KU/L
GLDH (mammal)	>12 KU/L
Adenosine diphosphate	5.0 mM
NADH analog[3]	0.20 mM

Buffer, pH 8 at 25ºC, preservative, and stabilizers

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagent contains less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8ºC. The reagent is stable on-board in the refrigerated reagent chamber of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear unhemolyzed serum and plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Serum BUN is stable for one day at 18 – 25ºC, several days at 2 – 8 ºC or six months at -15 ºC.[4] Since urea is susceptible to bacterial degradation, specimens should be stored at 2 – 8ºC until analysis.[5]

Limitations and Interfering Collection Tube Additives
Ammonium ions present in water or other substances may falsely elevate urea values. Avoid close proximity to a urinalysis laboratory or cleaning supplies containing ammonia.

PROCEDURE

Materials Provided
Medica BUN Reagent Wedge, REF 10202

Additional materials required

Medica EasyCal Chemistry, REF 10651

Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

INSTRUCTIONS FOR USE

The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (60 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the neck of the wedge for foam after removing the cap and placing the wedge on the EasyRA Analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test.

Calibration
Medica EasyCal Chemistry (REF 10651) is recommended for the calibration of the assay. The calibration interval (7 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the EasyRA Analyzer calculates the BUN concentration from the ratio of the change in the corrected unknown sample's absorbance per minute to the change in the corrected absorbance of the calibrator per minute multiplied by the calibrator value.

$$\text{BUN (mg/dL)} = \frac{(\Delta A/\text{Min}_{U} - \Delta A/\text{Min}_{Blk})_{340}}{[(\Delta A/\text{Min}_{C} - \Delta A/\text{Min}_{Blk})_{340}} \times \text{Cal Value}$$

Where $\Delta A/\text{Min}_U$ and $\Delta A/\text{Min}_C$ are the change in absorbance values per minute of the unknown and the calibrator respectively, $\Delta A/\text{Min}_{Blk}$ is the change in absorbance of the reagent blank, and "Cal Value" is the concentration of BUN in the calibrator (mg/dL).

Expected Values[1]
The reference range for BUN in serum and plasma is as follows:

Normal: 11 – 37 mg/dL

These values are guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
Only unhemolyzed serum or plasma samples should be used.

The EasyRA Analyzer flags any result above 70 mg/dL as Linearity High "LH. If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the BUN test to 140 mg/dL.

PERFORMANCE CHARACTERISTICS[6]

Reportable Range
The reportable range is 1 to 70 mg/dL Extended range is 1 to 140 mg/dL when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for BUN (y) on the EasyRA Analyzer utilizing a primary wavelength of 340 nm only to the performance of the same BUN reagent (x) on the EasyRA Analyzer utilizing a primary wavelength of 340 nm and a secondary wavelength of 700 nm. The data shown below represents single determinations obtained utilizing a primary wavelength and a secondary wavelength on the EasyRA Analyzer vs. the average of two replicate values obtained utilizing a primary wavelength on the EasyRA Analyzer.

Number of samples	48	Range of samples	2.0 to 67.0 mg/dL
Slope	1.0095	y Intercept	-0.0253
Correlation Coefficient	0.9993	Regression Equation:	Y = 1.0095*X - 0.0253

The following table lists the data obtained in a comparison of matched serum (x) and Li-Heparinized plasma (y) samples using the Medica Reagent for BUN on the EasyRA Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	71	Range of Samples	5.2 to 63.3 mg/dL
Slope	1.0028	y Intercept	-0.4871
Correlation	0.9989	Regression Equation	Y = 1.0028*X – 0.4871

**Cobas Mira is a registered trademark of Roche Diagnostics Operations, INC., Indianapolis, IN.*

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
48.7	0.3	0.6
31.1	0.2	0.7
12.2	0.2	1.6

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
48.7	0.5	1.1
31.1	0.4	1.2
12.2	0.2	1.8

Linearity (CLSI, EP6-A)
Linear from 1 to 70 mg/dL, based on the linear regression Y = 0.9992*X + 0.8761.

Limit of Blank (LOB):	0.5 mg/dL	(CLSI, EP17-A)
Limit of Detection (LOD):	0.8 mg/dL	(CLSI, EP17-A)
Limit of Quantitation (LoQ):	1.3 mg/dL	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as “no significant interference.”
There is significant positive interference to hemoglobin above 300 mg/dL. Do not use hemolyzed samples.
No significant interference was found in levels up to 40 mg/dL of bilirubin.
No significant interference was found in levels up to 800 mg/dL of triglycerides (using Intralipid*).
Samples from patients with Waldenstrom’s Macroglobulinemia has a high potential for interference and may produce unreliable results.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[7]

REFERENCES

1 Tietz, N.W., *Textbook of Clinical Chemistry*, W.B. Saunders Co., 3rd ed, p676, 1987.
2 Talke, H., Schubert, G.E., *Enzymatishche Harnstoffbestimmung in BLUT and Serum in Optishcen Test NACH Warburg*, Klin. Wchnschr 43, 174 (1965).
3 U.S. Patent No. 5,801,006.
4 Henry, RJ, Cannon DC, Winkleman, JW. *Clinical Chemistry: Principles and Techniques,* 2nd ed. Hagerstown, MD., Harper and Row; 1974:516.
5 Kaplan, L.A. and Pesce, A.J., *Clinical Chemistry – Theory, Analysis, and Correlation*, Third Edition. Mosby Year-Book Inc., St. Louis, p. 500 (1996).
6 Data on file at Medica
7 Young DS. Young's Effects on-line. *Effects of Drugs, Physiology, Preanalytical variables and herbs on Clinical Laboratory Tests*. AACC www.fxol.org

EasyRA Assay Parameters (BUN)

Primary Wavelength (nm)	340
Secondary Wavelength (nm)	700
Reaction Type	Kinetic (1)
Reaction Direction	Decrease
Reagent Blank	Yes (with each calibration)
Sample Blank	No
Max. first interval Abs. Change	0.20
Reaction Time	2.8 min
Calibration interval (maximum)	7 days
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	3.5
Diluent volume (µl)	0
Reagent volume (µl)	200
Decimal Places (default)	1
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	1 to 70 mg/dL

Medica Corporation, 5 Oak Park Drive
Bedford, Massachusetts 01730-1413 USA

EC REP

Emergo Europe, Westervoortsedijk 60
6827 AT Arnhem, The Netherlands