UREA-LS

INTENDED USE

Vitro urea/BUN reagent is intended for the in vitro quantitative determination of urea/BUN in serum, plasma and urine on both automated and manual systems.

METHOD

Enzymatic, colorimetric method (urease) modified Berthelot reaction.

CLINICAL SIGNIFICANCE

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver from ammonia, which is produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action. Determination of blood urea nitrogen is the most widely used screening test for renal function. When used in conjunction with serum creatinine determinations it can aid in the differential diagnosis of the three types of azotemia: prerenal, renal, and postrenal. Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, diminished blood volume (prerenal causes), chronic nephritis, nephrosclerosis, tubular necrosis, glomerular-nephritis (renal causes), and urinary tract obstruction (postrenal causes). Transient elevations may also be seen during periods of high protein intake. Unpredictable levels occur with liver diseases[1].

ASSAY PRINCIPLE

The measurement of urea nitrogen is performed primarily either by a condensation reaction using diacetyl monoxime or by enzymatic hydrolysis of urea by urease to produce ammonia. The diacetyl monoxime method was first proposed by Fearon[2] in 1939, and modifications of this colorimetric method are in wide use. The use of urease in BUN determinations was introduced by Marshall[3] who measured the liberated ammonia by titration with an acid. Ammonia produced by urease action has also been measured by nesslerization[4, 5] and by the Berthelot reaction[6]. Vitro Urea/BUN endpoint reagents use the modified Berthelot reaction. The series of reactions involved in the assay system are as follows:

1. Urea is hydrolyzed by urease to form ammonium and carbonate.
2. In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored indophenol (2.2 dicarboxylindophenol).

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

$$2NH_3 + \text{Salicylate} + \text{Hypochlorite} \longrightarrow \text{2,2-Dicarboxyindophenol}$$

The intensity of the color produced is directly proportional to urea/BUN concentration. It is determined by measuring the increase in absorbance at 578 – 630 nm.

EXPECTED VALUES

Serum or plasma[7]	
Urea	15 – 45 mg/dl 2.5 – 7.5 mmol/l
BUN	7.0 – 21 mg/dl 5.11– 15 mmol/l
Urine[7]	
Urea	20 - 35 g/day 0.33 – 0.58 mol/day
BUN	9.0 - 17 g/day 0.66 – 11.6 mol/day

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the Urea/BUN results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Urea standard	50	mg/dl
R_2	Phosphate buffer, pH 8.0	40	mmol/l
	Sodium salicylate	52	mmol/l
	Sodium nitroprusside	1	mmol/l
	EDTA	1	mmol/l
	Urease	>5000	U/l
R_3	Sodium hypochlorite	10	mmol/l
	Sodium hydroxide	20	mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMEN

- Serum, plasma, and urine
- Don't use ammonium heparin.

Specimen Preparation & Stability

- **For serum or plasma specimen**

No special preparation of the patient is necessary. Bacterial growth in the specimen and high atmospheric ammonia concentration as well as contamination by ammonium ions may cause erroneously elevated results. Urea remains stable in serum samples for 24 hours at room temperature, for several days at 4^{0}C, and for at least 2-3 months when frozen [1, 7].

- **For urine specimen**

For 24 hours collections add thymol as preservative to prevent bacterial degradation to the container before collection. Urea in urine is stable for 4 days at room temperature[8]. Urine specimens diluted 1:100 (1+99) with water prior to analysis.

PROCEDURE

- **Manual Procedure**

Wavelength	578 - 630 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum, plasma or urine

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Pre-warm at 37^{0}C for one minutes if incubation temperature of the assay 37^{0}C.			
Standard	……	10 µl	……
Specimen	……	……	10 µl
Mix, incubate for 3 minutes at 37^{0}C or 5 minute at 20-25^{0}C			
R_3	200 µl	200 µl	200 µl

Mix, incubate for 5 minutes at 37^{0}C or 10 minute at 20-25^{0}C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 120 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the urea concentration by using the following formulae:

$$\text{Urea Concentration} = \frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

- **Unit conversion**

mg/dl x 0.166 = mmol/l
BUN = Urea / 2.14

~~For urine specimen~~ ~~by the dilution factor~~ and 24 hours collections by the volume in liters.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com

EC REP	Medical Device Safety Services MDSS GmbH, Burckhardtstr. 1 30163 Hannover, Germany.

- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established urea values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.

- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Don't use ammonium heparin as an anticoagulant.
- **Bilirubin:**
  No interference from free bilirubin up to level of 19 mg/dl and from conjugated bilirubin up to a level of 18 mg/dl.
- **Drugs:**
  Young[9] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.
- **Haemoglobin:**
  No significant interference from haemoglobin up to a level of 522 mg/dl. Haemolysed specimens may cause high absorbance flagging.
- **Lipemia:**
  No significant interference. Lipemic specimens may cause high absorbance flagging
- **Others:**
  Ammonium ions may cause erroneously elevated results.

WARNING & PRECAUTION

- Vitro urea reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid.
- Specimens must be drawn in a soap and ammonium ions free collection device.
- Because of urea's susceptibility to bacterial contamination, it is recommended that all specimens be stored refrigerated at 4^0C until analysis.
- Don't expose the reaction medium to direct strong light.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	40	40	40	40
Mean (mg/dl)	19	57	19.3	58.5
SD (mg/dl)	0.55	0.58	0.8	0.65
CV (%)	3.6	1.2	3.8	3.8

Method Comparison

Comparison studies were carried out using a similar commercially available reagent as a reference. Serum and urine samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs 45
Range of results 11 - 66 mg/dl
Mean of reference method results19 mg/dl
Mean of Infinity Urea results 19 mg/dl
Slope 0.98
Intercept 0.18 g/dl
Correlation coefficient 0.989

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.
When run as recommended the sensitivity of this assay is 0.2 mg/dl (0.033 mmol/l).

LINEARITY

When run as recommended, the assay is linear up to 200 mg/dl (33.3 mmol/l).
If result exceeds 200 mg/dl (33.3 mmol/l), specimen should be diluted with ammonia free water and reassayed. Multiply the result by the dilution factor.
It is possible to use 1 ml of diluted R_4 (1:5 with distilled water) by this procedure; the assay is linear up to 300 mg/dl (50 mmol/l).

BIBLIOGRAPHY

1. **Rock, RC, Walker, WG & Jennings, CD (1987):** Nitrogen metabolites and renal function. In: Tietz NW, ed. Fundamentals of clinical chemistry. 3rd ed. Philadellphia: WB Saunders; 669-704.
2. **Fearon, WR (1939):** Biochem. J. 331:902.
3. **Marshall, EK (1913):** J. Biol. Chem. 151:487.
4. **Gentzkow, CJ (1942):** J. Biol. Chem. 143:531.
5. **Karr, WB (1924):** J. Lab. Clin. Med. 9:329.
6. **Fawcett, JK & Scott, JE (1960):** J. Clin. Pathol. 13:156.
7. **Richterich, R, & Colombo, JP (1978):** Klinische Chemie. 4th ed. Basel: Karger S; 319.
8. **Tietz, NW, ed (1990):** Clinical Guide to laboratory Tests. 2nd ed. Philadelphia: WB Saunders; 566.
9. **Young, DS (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
	Batch code (Lot #)
	Catalog number
	Temperature limitation
	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1 x 100 ml	14101
2 x 100 ml	14102

Article # :141-EN
Date of Revision : 03/2021

ISO 13485:2016 CE

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com

EC REP **Medical Device Safety Services**
MDSS GmbH, Burckhardtstr. 1
30163 Hannover, Germany.