LiquiMAX Urea

Modified Berthelot Method

ORDERING INFORMATION

Ref. No:	Pack Size	Presentation
AVURE3-100	2 x 50 ml	Two Liquid Reagents
AVURE3-200	2 x 100 ml	and Enzyme
AVURE3-400	4 x 100 ml	Concentrate

INTENDED USE:

LiquiMAX Urea is an in-vitro diagnostic kit is use for the quantitative determination of urea in human serum, plasma and urine.

PRODUCT FEATURES

1. Liquid Stable, Ready to use Two Reagents with Enzyme Concentrate
2. Working reagent stability of 12 months at 2-8°C.
3. 8 Minutes End Point Assay (3 Min + 5 Min)
4. Lipid Clearing Factor
5. Linearity 350 mg/dl
6. Measuring Wavelength 578 nm (570-620)
7. Aqueous Urea Standrad provided (Standrad Conc: 50 mg/dl)
8. BUN values can be estimated
9. Serum/ Heparinized or EDTA Plasma/ Diluted Urine as specimens
10. Available as multipurpose reagents.

CLINICAL SIGNIFICANCE:

The determination of urea is the most widely used test for the evaluation of kidney function. The test is frequently used in conjunction with the determination of creatinine for the differential diagnosis of prerenal hyperuremia (cardiac decompensation, water depletion increased protein catabolism), renal hyperuremia (glomerulonephritis, chronic nephritis. polycystic kidney, nephrosclerosis, tubular necrosis) and postrenal hyperuremia (obstructions of the urinary tract). Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is metabolized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body. In 1914 Marshall introduced an assay based on the enzyme urease for determining urea in blood. The ammonia released from urea by urease was measured titrimetrically. Numerous other techniques have since been employed to measure the ammonia produced. These include Berthelot's indophenol assay and the reaction of ammonia with Nessler's reagent.

PRINCIPLE:

Urease catalyses the conversion of Urea to Ammonia and Carbondioxide. The ammonia released reacts with a mixture of Salicylate, Hypochlorite and Nitroprusside to yield a blue-green colored compound (Indophenol).

The intensity of color produced is proportional to the concentration of urea in the sample and is measured photometrically at 578 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + Co_2$$

$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \xrightarrow{\text{Nitroprusside}} \text{2 - 2 - Dicarboxy Indophenol.}$$

STORAGE & STABILITY:

All the reagents must be stored at 2-8°C and are stable till the expiry date mentioned on the labels.

KIT COMPONENTS

1. Urease Reagent R1
2. Enzyme Reagent R1A
3. Color Reagent R2
4. Urea Standard : Concentration as stated on the label

COMPOSITION:

Urease = 40,000 U/l
Sodium Hypo chlorite =30 mMol/l
NaOH = 380 mMol/l
Sodium Salicylate = 50 m mol/l
Sodium Nitroprusside = 28 m mol/l
Activators and Stabilizers

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

1. When the reagent is stored properly at 2-8ºC & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.
2. Transfer the entire Enzyme Concentrate (1A) into Urease Reagent (1 with the Micro Pipette).
3. After transferring the Enzyme Concentrate in to the Urease Reagent rinse the Enzyme Concentrate Vial thoroughly at least three times with Urease Reagent and take the left over enzyme completely to ensure proper reconstitution as the enzyme is provided in the highly concentrated form.
4. The reconstituted reagent is stable for 12 months when proper storage conditions are strictly maintained.
5. Avoid keeping the reconstituted reagent at room temperature for a long time.
6. It is advised to keep the reagent back at 2-8°C once the assay is over.
7. Slight haziness / turbidity may appear in the Enzyme concentrate and is only due to high concentration of enzyme. Slight haziness / turbidity of enzyme concentrate disappears once it is added to Urease Reagent and does not affect test performance and results.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent if blank reagent absorbance exceeds 0.2 at 578 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN:

Serum / Heparinised or EDTA Plasma (do not use ammonium salts and sodium fluoride as anticoagulants) Urine (dilute 1:100 with distilled water).

SYSTEM PARAMETERS:

Reaction type	:	End Point
Flow cell Temp	:	37°C
Units	:	mg/dl
Reagent Volume	:	2000 µl (1000 µl R 1, 1000 µl R 2)
Sample Volume	:	10 µl
Wavelength	:	578 nm (570-620 nm)
Blank	:	Reagent
Standard Conc	:	50
Linearity	:	350
High Normal	:	50
Low Normal	:	10

TEST PROCEDURE:

Reagent	Blank	Standard	Test
Working Reagent	1000 µl	1000 µl	1000 µl
Urea Standard (Conc. 50 mg/dl)	----	10 µl	----
Specimen	----	----	10 µl
Mix and incubate for 3 minutes at 37°C (5 min. at R.T.)			
Colour Reagent	1000 µl	1000 µl	1000 µl
Mix and incubate for 5 minutes at 37°C (10 min. at R.T.)			

Mix and read absorbance of Standard (S) and Test (T) against Blank (B) at 578 nm (570-620 nm).
The final color is stable for 10 hours at RT.

CALCULATIONS:

a) Serum / Plasma Urea in mg/dl = $\frac{\text{Abs. of T}}{\text{Abs. of S}}$ x 50

b) Blood Urea Nitrogen (BUN) in mg/dl = a x 0.467

c) Urine Urea in gm / 24 hours = a x 24hrs urine volume in litres
Urine UREA/BUN in gm/24hours = Conc. of UREA in gm/L x 24 hours Urine Collected in Liters.

Estimantion of UREA /BUN in Urine (gm/24 hours) Procedure

Measure and record 24 hrs urine volume collected in liters.
Determine the UREA/ BUN Conc. in mg/dl using LiquiMAX Urea(Berthelot) kit.
Convert the UREA/BUN Conc. into mg/L by multiplying with factor "10".
Convert the UREA/BUN Conc. from mg/L to gm/L by dividing with "1000".
Multiply the UREA/BUN conc. which is in gm/L with 24 hrs urine collected in

EXPECTED VALUES

Serum / Plasma Urea : 10-50 mg/dl
Urine Urea : 25-43 gm/24 hrs
Serum / plasma Urea Nitrogen : 5-23 mg/dl

It is recommended that the laboratories should establish their own normal range.

QUALITY CONTROL & CALIBRATION

It is recommend to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result. Using the recommended calibrator (Avecon) or the standard included, calibrate the assay:
a) When using a new reagent or lot.
b) When QC values are out of range.

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 350 mg/dl

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 0.2 mg/dl

3. Interferences

No significant interference was observed from Bilirubin up to 20 mg/dl (Both conjugated and unconjugated Bilirubin) Hemoglobin up to 50 mg/dl,Lipemia as Triglycerides up to 2000 mg/dl, Ascorbic acid up to 50 mg/dl.

4. Precision:

Reproducibility was determined using human samples and controls between day (n = 18). The following results were obtained:

Sample N=18	Intra-Assay Mean mg/dl	SD mg/dl	CV %
Sample 1	39.61	0.88	2.21
Sample 2	90.95	5.65	6.21
Sample 3	139.78	1.95	1.40

Sample N=18	Inter-Assay Mean mg/dl	SD mg/dl	CV %
Sample 1	39.85	1.52	3.81
Sample 2	89.48	3.47	3.87
Sample 3	140.20	5.27	3.76

5. Method Comparison:

A comparison of the LiquiMAX Urea Berthelot (y) with a commercial obtainable assay (x) gave the following result : y = 1.113 x – 0.278; r = 0.990

LIMITATIONS

Measuring range: 0.2-350 mg/dl. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

NOTES:

1. Urease Reagent is pale yellow in color due to which the Blank absorbance may read upto 0.200 at 578 m against distilled water. The absorbance of Standard and Test read against Reagent Blank at 578 nm nullifies the absorbance of Urease Reagent.
2. Contamination of reagents and standard after opening must be avoided. After use, all the reagents must be immediately stored back at 2-8°C. Working Reagent is stable for 12 months at 2-8°C.
3. No interference was observed by ascorbic acid up to 32 mg/dl, bilirubin up to 43 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2200 mg/dl triglycerides.
4. If the urea value exceeds 350 mg/dl, dilute sample with normal saline. In such a case, the result obtained must be multiplied with the appropriate dilution factor.
5. Working reagent preparation is a very important step and the instructions need to be followed Strictly.

REFERENCES:

- Chaney, A.L. and Marbach, E.P. (1962) Clin. Chem. 8, 130
- Tietz NW. Fundametals of Clinical Chemistry Philadelphia, Pa: WB Saunders Co 1976:991 .

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

Manufactured in India by :
AVECON Healthcare Pvt. Ltd.
Transforming Research into Innovations
Plot No.: 338, Sector-2, Industrial Growth Centre, Saha, Ambala, Haryana (INDIA)-133104
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