LiquiMAX UREA - UV

UREASE-GLDH Method

ORDERING INFORMATION

REf. No	Pack Size	Presentation
AVURE2-100	4 x 25 ml	Two Liquid Reagents and Standard
AVURE2-250	10 x 25 ml	
AVURE2-500	20 x 25 ml	

INTENDED USE:

LiquiMAX UREA-UV 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.
2. Incorporates 5 th Gen NADH Analogue.
3. 3 Minutes Fixed Time Assay (60 Sec Delay + 120 Sec Measuring)
4. Linearity 300 mg/dl
5. Measuring Wavelength 340 nms
6. Aqueous Urea Standrad provided (Standrad Conc: 50 mg/dl)
7. BUN values can be estimated
8. Serum/ Heparinized or EDTA Plasma/ Diluted Urine as specimens

CLINICAL SIGNIFICANCE :

Urea is a metabolic product derived sequentially from the catabolism of either exogenous or endogenous tissue proteins. It is the major nitrogen containing metabolic product of protein catabolism in humans accounting for more that 75% of the non-protein nitrogen eventually excreted. Urea is typically measured in conjunction with creatinine to differentiate between pre-renal and post-renal uraemia. Pre-renal uraemia is observed in cardiac de-compensation, water depletion and increased protein catabolism. Post-renal uraemia is observed in glomerular nephritis, chronic nephritis, polycystic kidney and nephrosclerosis.nt.

PRINCIPLE

Urea is converted in the presence of urease to ammonia. Ammonia is then linked with alpha ketoglutarate in the presence of glutamate dehydrogenase (GLDH) with the subsequent conversion of NADH Analogue to an NAD. The rate of NADH Analogue consumption is directly proportional to the urea concentration in the patient sample. Enzymatic determination according to the following reactions:

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

$$2NH4 + 2\alpha\text{-Ketoglutarate} + 2NADH\,ANALOGUE+ \xrightarrow{\text{GLDH}} 2\,\text{Glutamate} + 2NAD+ + 2H_2O$$

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. Buffer Reagent R1
2. Substrate Reagent R2
3. Urea Standard : Concentration as stated on the label

COMPOSITION:

Component	Ingredients	Concentration in Tests
Reagent 1	TRIS Buffer	pH 7.95 112 mmol/l
	α-KG	15.5 mmol/l
	ADP	0.94 mmol/l
	Urease	17000 U/l
	GLDH	600 U/l
	PRESERVATIVES & STABILISERS	
Reagent 2	α-Ketoglutarate	115 mmol/l
	NADH	1.44 mmol/l
	PRESERVATIVES & STABILISERS	
Standard	Urea	8.35 mmol/l

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

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 or blank reagent absorbance less than 0.8 at 340 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 COLLECTION AND STORAGE

Use serum, heparin or EDTA plasma as specimen (do not use ammonium heparin). Collect urine without using preservatives.

It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum/plasma should be separated from cells within 2 hours after collection. Stability up to 7 days at 4°C.

Urine should be diluted 1:20 with distilled water. Multiply results obtained by dilution factor. Stability up to 7 days at 4°C

SYSTEM PARAMETERS:

Reaction Type (Mode)	:	Fixed Time
Reaction Direction	:	Decreasing
Wave Length	:	340 nm
Flow Cell Temp.	:	37°C
Zero Setting with	:	Distilled Water
Delay Time	:	60 Seconds
Measuring Time	:	120 Seconds
Working Reagent Volume	:	1.0 ml
Standard / Sample Volume	:	10 µl
Units	:	mg/dl
Standard Concentration	:	50
Linearity	:	300
High Normal	:	50
Low Normal	:	10

TEST PROCEDURE:

Reagent	S	T
Reagent-1	800 µl	800 µl
Urea Standard (Conc. 50 mg/dl)	10 µl	----
Specimen	----	10 µl
Reagent-2	200 µl	200 µl

Gently mix and aspirate in to the analyzer. Measure the change in Optical Density (ΔOD/min) between 60 Seconds(A1) and 180 seconds (A2) in a Fixed Time Programme at 340 nms

CALCULATIONS:

(a) Serum / Plasma Urea in mg/dl = $\frac{\text{Delta Abs. of T}}{\text{Delta Abs. of S}}$ 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-UV FT 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 liters to get the UREA/BUN Conc. in gm/24hrs.

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 : 300 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:

Serum
Reproducibility was determined using human samples and controls between day (n = 20). The following results were obtained:

Intra-Assay			
Sample N=20	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

Inter-Assay			
Sample N=20	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 UV (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.

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		

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GMP 5th Schedule MDR 2017

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