UREA AND UREA NITROGEN

(DAM) Colorimetric End Point

BIO LAB DIAGNOSTICS
ISO 9001:2015
ISO 13485:2016
CE

CLINICAL SIGNIFICANCE

Increased levels are associated with renal diseases as well as dehydration, diabetic coma, hypoadrenal crisis, gastrointestinal haemorrhage and circulatory collapse. Decreased values are observed in some cases of severe liver diseases.

PRINCIPLE

In the presence of ferric ions and in a warm acid medium urea reacts with Diacetylmonoxime to produce a Pink coloured compound. The intensity of this colour is proportional to urea concentration. Thiosemicarbazide catalyses this reaction and helps avoid deproteinization of serum.

REAGENTS COMPOSITION

1.	**Acid Reagent**	**100 mL**
	Thiosemicarbazide	14 mMol/L
	Ferric Chloride	55 mMol/L
	Sulphuric Acid	3.6 Mol/L
	Phosphoric acid	45 mMol/L
2.	**DAM Reagent**	**100 mL**
	Diacetylmonoxime	66 mMol/L
3.	**Standard**	**5 mL**
	Urea	40.00 mGs/dL
	Urea Nitrogen	18.65 mGs/dL

Working Reagent Preparation

All Reagents are ready to use.

STORAGE AND STABILITY

When stored at 2-8°C and protected from light, the reagents are stable until the expiry date stated on the label.

SAMPLE

Sample can be serum or plasma which has no sign of haemolysis. Common anticoagulants have no interference on this assay. If Urine sample is to be tested it must be diluted 1 to 100 and the result is multiplied by 100 or dilution factor. (All samples should be handled as potential infective agents as no laboratory methods make conclusive findings for its safety. Therefore, adequate protective laboratory measures should be taken while handling such materials).

MANUAL METHOD

Keep water bath ready and water boiling before the test. Avoid cross contamination of reagents by using separate pipettes. Keep test tubes in boiling water bath immediately on addition of DAM reagent (Reagent No.2)

Pipette into 3 Test Tubes	**BLANK mL**	**STD mL**	**TEST mL**
Reagent No 1	1.50	1.50	1.50
Standard Reagent No 3	-	0.02	-
Sample	-	-	0.02
Mix carefully.			
Add Reagent No.2	1.50	1.50	1.50

Mix well. Immediately keep in the boiling water bath for exactly 10 minutes. Cool for 5 minutes under running tap water. Read at 540 nM (510-570 nM) or GREEN filter against Blank. Final colour is stable for 30 minutes.

RESULTS

$$\text{Urea in mGs/dL} = \frac{\text{OD Test - OD Blank}}{\text{OD STD - OD Blank}} \times 40$$

BUN = Urea mGs/dL x 0.466
mMol/L = Urea mGs/dL x 0.1665

Example:
OD of Blank = 0.01 and OD of Test = 0.20
OD Standard = 0.38

$$\text{Urea in mGs/dL} = \frac{0.20 - 0.01}{0.38 - 0.01} \times 40 = 20.54$$

EXPECTED VALUES

Serum or Plasma Urea	10-40 mGs/dL
BUN	(4.6-18 mGs/dL)
Urine Urea	20-35 Gms/24 hrs

As with all diagnostic methods, the final diagnosis should not be made on the result of a single test as well as laboratory diagnosis must be confirmed with clinical manifestations.

LIMITATIONS

This reagent system requires protein precipitation in case of samples having hyper-macroglobulinamea to avoid turbidity with working reagent resulting in false reporting and formation of potential mass of precipitated protein in the flow cell. Therefore, such sample should be pre-identified and treated with suitable protein precipitants.

This assay is linear upto 80 mGs/dL serum urea or 37 mGs/dL BUN. For values higher than this limit, dilute the sample with distilled water, free from ammonium salts. Rerun the assay and multiply the result with dilution factor i.e. by 2 for 1:1 dilution.

WARNING

This reagent system is for ***invitro*** use only. This reagent system is containing preservatives and components that have not established for safety, if contacted on broken skin or eye or taken orally, in case of such incidents wash off with plenty of water, or consult a physician.

QUALITY CONTROL

To ensure adequate quality control, each kit should be tested against a standard control sera. It should be realised that the use of quality control material checks both instrument and reagent function together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glasswares and accuracy of pipetting.

It is appropriate to establish each laboratory's accuracy constant and interpret values. Similarly, laboratory findings should be established by clinical manifestations.

BIBLIOGRAPHY

Bousquet B., F. Et J., Julien r., Bon R., Dreux C. Ann Biol. Clin., 1971, 29, 415. Marsh W. H., Fingerhut B., Miler H., Clin. Chem 1965,11, 624.

BIOLAB DIAGNOSTICS (I) PVT. LTD.
J-245, MIDC, TARAPUR, BOISAR - 401 501, MAHARASHTRA.
E-mail : biolab@vsnl.com / www.biolabdiagnostics.com
Customer Care : (+ 9122) 28088243