

Intended Use

The reagents are used for the quantitative determination of Urea in serum or plasma. For in-vitro diagnostic use only.

Introduction

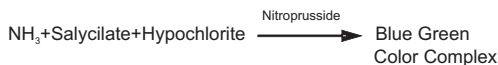
Urea is produced in the liver, as a waste product in the urea cycle from the catabolism of proteins in humans. Consequently, the circulation levels of urea depend upon protein intake, protein catabolism and kidney function. Low levels of urea are not common. It can be seen in severe liver disease or malnutrition but are not used to diagnose or monitor these conditions. Low urea level also observed in normal pregnancy. Elevated levels of urea suggest impaired kidney function but it may also be due to congestive heart failure, shock, recent heart attack or severe burns, bleeding from the gastrointestinal tract and conditions that cause obstruction of urine flow or dehydration.

Method

Berthelot method - End Point.

Principle

Urease catalyzes the conversion of urea into ammonia and CO₂. Ammonia released reacts with a salicylate in presence of a nitroprusside and hypochlorite, giving a blue green colored complex.



Absorbance of the colored solution is directly proportional to the urea concentration, when measured at 578nm.

Reagent Composition

Reagent 1:

Sodium Salicylate	60.02 mmol/L
Sodium Nitroprusside	5 mmol/L

1

Reagent 2:

Urease	> 4 U/ml
--------	----------

Reagent 3:

Hypochlorite solution (4% Chloride w/v)	20 ml/L
---	---------

Reagent 4:

Urea Standard	40 mg/dl
---------------	----------

Precautions

Following precautions should be taken:

- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.
- Keep the bottles tightly closed after use.

Reagent Storage and Stability

- Unopened Reagents are stable till expiry mentioned on the label when stored at 2 – 8°C temperature.
- Reagent 3 is stable for 6 months after opening the bottle.
- Standard Reagent R4 is stable till expiry mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 5 (Urea: 150mg/dl) & Reagent 6 (Urea: 250mg/dl) can be provided for linearity check with Reagent 4 (Urea: 40mg/dl-Standard) .

Reagent Preparation

Reagents are ready for use.

Reagent Deterioration

Reagents should be clear . Turbidity and/or precipitation may be because of deterioration.

Sample Collection and Storage

Unhaemolysed serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. Do not use ammonium salts and sodium fluoride as anti-coagulants. It is recommended to use freshly collected samples for assay. Serum samples can be stored for 72hrs at 2-8°C.

2

For urine sample 1:20 dilution with ammonia free water is required before analysis. Urine samples to be tested within 8hr.

General Assay Parameters

Mode	End Point
Wavelength 1 (nm)	578
Wavelength 2 (nm)	-
Blank with	Reagent
Sample Volume (µl)	5/10
Working solution(µl)	500/1000
Incubation Time 1 (min)	3
Hypochlorite solution(µl)	500/1000
Incubation Time 2 (min)	5
Incubation Temperature(°C)	37
Normal Low (mg/dl)	11
Normal High (mg/dl)	44
Linearity (mg/dl)	Upto 250
Standard Conc. (mg/dl)	40
Units	mg/dl

Procedure

Preparation of working solution

For multiple number of assay, prepare the working solution by mixing ten parts of the Reagent 1 and one part of the Reagent 2. This solution is stable for 4 weeks at 2-8°C.

Pipette into test tube as given below:

Particulars	Blank	Standard	Sample
Working soln.	1000 µl	1000 µl	1000 µl
Reagent 4	-	10 µl	-
Sample			10 µl
Mix and incubate exactly for 3 min. at 37°C. Then add:			
Reagent 3	1000 µl	1000 µl	1000 µl

3

Mix and incubate exactly for 5 min. at 37°C. Read absorbance of sample and absorbance of standard against blank.

Calculation

Urea concentration in the sample can be calculated using the following formula:

$$\text{Urea (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

$$\frac{0.200}{0.180} \times 40 = 44.44 \text{ mg/dl}$$

If the urea concentration exceeds 250mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

Serum:

Urea : 11-44mg/dl

Urea Nitrogen : 5-21mg/dl

Urine:

Urea : 20-35g/24hr

Limitations

- 1.The reagent and sample volumes can be altered proportionately so that the sample:reagent ratio remains same.
- 2.Hemolytic and lipemic samples may result in falsely elevated results. To avoid false results sample blank may be used as mentioned below:

•Add 10µl of serum sample to 1000µl of DI water and read absorbance at 578nm.

•Subtract the absorbance obtained as above, from the absorbance of test. Use this corrected absorbance for calculation.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for urea.

Performance

Linearity Limit: 250 mg/dl

Precision:

Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	31.03	95.39
S.D.	0.58	1.05
C.V. %	1.88	1.10

Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	31.50	95.58
S.D.	0.84	1.64
C.V. %	2.66	1.71

References

1. Nicolaou, Kyriacos Costa; Tamsyn Montagnon (2008). Molecules That Changed The World.Wiley-VCH. pp. 11. ISBN 978-3-527-30983-2.<http://en.wikipedia.org/wiki/Urea>
2. <http://www.labtestsonline.org.uk/understanding/analytes/urea/test.html>
3. Tietz NW. Clinical guide to laboratory tests, 2nd ed. Saunders Co., 1991.
4. Allain CC, Poon LS, Chan CSG, Richmond W and Fu PC. Enzymatic determination of total serum Urea. Clin Chem 1978; 20: 470 – 475.
5. Fawcett, J.K., Scott, J.E; K. Clin. Path., 1960, 13, 156-159.
6. Weatherburn, M.W.; Anal. Chem. 1967, 39, 971-974.

Pack Presentation

Product Code/ Catalogue No.	KGURE101.4.1	KGURE101.4.2
Pack Size *	2x 100 ml	4x 100 ml
Reagent 1	1X100 ml	2x 100 ml
Reagent 2	1X10 ml	1x 20 ml
Reagent 3	1X100 ml	2x 100 ml
Reagent 4	1X2 ml	1x 3 ml

*Pack size may vary on market demand.

Revision No: (Ver: KGURE101.4/1)

Date of Issue: 1st April 2010.

Symbols

Following symbols are used in the labeling of KEE GAD kits:



Catalogue No.



Batch No.



CE Mark



Read instructions



In Vitro Diagnostics



Storage temperature



Expiry Date



Content



Product Name



Manufactured By



Manufactured by:

KEE GAD Biogen Pvt. Ltd.

A-8, Third Floor, Naraina Industrial Area,
Phase-II, New Delhi-110028 (India)