QUADRAPED™

UREA KIT

(GLDH Kinetic Method)

(For veterinary invitro diagnostic use only)

INTENDED USE

QUADRAPED™ Urea kit is used for the determination of Urea in serum or plasma.

SUMMARY

Urea is the end product of the protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy.

PRINCIPLE

Urease hydrolyzes urea to ammonia and CO_2. The ammonia formed further combines with α Ketoglutarate and NADH to form Glutamate and NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance in a fixed time, which is proportional to the urea concentration in the sample.

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\textit{Urease}} 2NH_4^+ + CO_2$$

$$2NH_4^+ + 2\alpha\text{ Ketoglutarate} + 2\text{ NADH} \xrightarrow{\textit{GLDH}} 2\text{ L-glutamate} + 2NAD^+ + 2H_2O$$

EXPECTED VALUES

Species	Urea (mg/dl)
Dog	8- 30
Cat	13-32
Cow	17-30
Horse	21-51
Pig	21-49
Sheep	21-53
Goat	21-50
Rabbit	21-65
Buffalo	17-30

It is recommended that each laboratory establish its own range as reference ranges may vary between laboratories.

PRESENTATION

REF	1126220025
Pack Size	25 ml
L1 Enzyme Reagent	20 ml
L2 Starter Reagent	5 ml
S Urea Standard (40 mg/dl)	5 ml

COMPOSITION

Tris Buffer 100mM; pH 7.8; Urease > 7500U; GLDH > 1000U; NADH 0.18mM; Ketoglutarate 7mM; Non Reactive Stabilizers, Detergent and Preservative.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use.

Working reagent: For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 10 days when stored at 2-8°C.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Allow the working reagent to stand for 30 mins. at R.T. before use.

SAMPLE MATERIAL

Serum or plasma. Urea is reported to be stable in sample for 5 days at 2-8°C.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Wavelength / filter : 340 nm
Temperature : 37° C / 30° C / 25° C
Light path : 1 cm

Substrate Start Assay:

Pipette into clean dry test tubes labelled as Standard (S) or Test (T):

Addition Sequence	(S) / (T) 37° C / 30° C / 25° C
Enzyme Reagent (L1)	0.8 ml
Urea Standard / Sample	0.01 ml
Incubate at the assay temperature for 1 min. and add	
Starter Reagent (L2)	0.2 ml

Mix well and read the initial absorbance A_1 for the Standard and Test after exactly 30 secs. Read another absorbance A_2 of the Standard and Test exactly 60 secs. later. Calculate the change in absorbance ΔA for both the Standard and Test.

Sample Start Assay:

Pipette into clean dry test tubes labelled as Standard (S) or Test (T):

Addition Sequence	(S) / (T) 37° C / 30° C / 25° C
Working Reagent	1.0 ml
Bring to assay temperature and add	
Urea Standard / Sample	0.01 ml

Mix well and read the initial absorbance A_1 for the Standard and Test after exactly 30 secs. Read another absorbance A_2 of the Standard and Test exactly 60 secs. later. Calculate the change in absorbance ΔA for both the Standard and Test.

For Standard $\Delta AS = A_2S - A_1S$
For Test $\Delta AT = A_2T - A_1T$

CALCULATIONS

$$\text{Urea in mg/dl} = \frac{\Delta AT}{\Delta AS} \times 40$$

QUALITY CONTROL

The following process is recommended for QC during the assay of Urea. *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

LOD : 1.00 mg/dl
Lower Limit : 1.00 mg/dl
Higher Limit: 250 mg/dl

If the values exceed this limit, dilute the serum with normal saline (Nacl 0.9 %) and repeat the assay. Calculate the value using the proper dilution factor.

Interferences:

Sample when spike with interferent such as upto 30 mg/dl. Bilirubin,2000 mg/dl intralipid and 0.75 mg/dl. Haemoglobin does not affect the ability of the kit to determine the urea concentration.

Precision:
Within run

Within run	n	Mean	SD	% CV
Sample 1	10	47.68	0.47	0.98
Sample 2	10	124.8	0.45	0.36
Sample 3	10	16.5	0.14	0.87

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	47.53	0.65	1.37
Sample 2	10	124.1	0.74	0.59
Sample 3	10	16.4	0.14	0.79

Method comparison:

Comparative studies were done to compare our reagent with another commercial Urea Assay. No significant differences were observed. Details of the comparative studies are available on request.

NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. The reagent contain sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. Only clean and dry glassware must be used. Plasma should not be collected with Fluoride or Heparin salts as contamination by ammonia or ammonium salts lead to erroneous results. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Fawcett J.K. Scott J.E. (1960) J. Chim. Pathol. 13 : 156.
2. Chaey A., (1962) Clin. Chem. 8 : 130.
3. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Kenneth S. Latimer, ISBN Jane Wardrop , 6th Edition - 2010.
4. Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
5. Data on file: Coral Clinical Systems.

System Parameters

Reaction	: Fixed Time Kinetic	**Interval**	: 60 Sec.
Wavelength	: 340 nm	**Sample Vol.**	: 0.01 ml
Zero Setting	: Distilled Water	**Reagent Vol.**	: 1.00 ml
Incub. Temp.	: 30° C / 37° C	**Standard**	: 40 mg/dl
Incub. Time	: ---	**Factor**	: ---
Delay Time	: 30 Sec.	**React. Slope**	: Decreasing
Read Time	: 60 Sec.	**Linearity**	: 250 mg/dl
No. of read.	: 2	**Units**	: mg/dl

SYMBOL KEYS

8°C / 2°C Store at 2-8°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	L1 Enzyme Reagent	GLDH Kinetic — GLDH Kinetic Method
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	L2 Starter Reagent	S Urea Standard (40 mg/dl)
Date of Manufacture	REF Catalogue Number	This way up		

Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

BUILDING E, PLOT NO. M-46/47, PHASE III B, VERNA INDUSTRIAL ESTATE, VERNA, GOA-403 722, INDIA.

REGD. OFFICE : GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA.

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