Infinity™
Urea Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until expiry at 2-8°C
Linear Range	:	0.5 - 40 mmol/L (3 - 112 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Fixed Rate
Reagent Preparation	:	Supplied ready to use.

IVD Rx ONLY

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		
	Consult instructions for use		Manufactured by
Rx ONLY	Prescription Use Only		

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Urea (or Urea Nitrogen) in human serum, plasma or urine.

CLINICAL SIGNIFICANCE[1]
Urea is the major end product of protein nitrogen metabolism in humans. It constitutes the largest fraction of the non - protein nitrogen component of the blood. Urea is produced in the liver and excreted through the kidneys in the urine. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur with dietary changes, diseases which impair kidney function, liver disease, congestive heart failure, diabetes and infection.

METHODOLOGY
The enzyme methodology employed in this reagent is based on the reaction first described by Talke and Schubert.[2] To shorten and simplify the assay, the calculations are based on the discovery of Tiffany, et al.[3] that urea concentration is proportional to absorbance change over a fixed time interval.

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

$$NH_3 + \text{a-KG} + \text{NADH} \xrightarrow{GLDH} \text{L-Glutamate} + \text{NAD}$$

1. Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide.
2. In the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH), the ammonia combines with a-ketoglutarate (a-KG) to produce L-glutamate.

The Infinity Urea reagent also incorporates a patented stabilization process. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm as NADH is converted to NAD.

REAGENT COMPOSITION

Active Ingredients	Concentration
α-Ketoglutarate	7.5 mmol/L
NADH	> 0.20 mmol/L
Urease (Jack Bean)	> 5,000 U/L
GLDH (Micro-organism)	> 450 U/L
Tris buffer	100 mmol/L

Also contains non-reactive fillers and stabilisers.
pH 8.50 ± 0.1 at 20°C

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Infinity Urea Liquid Stable Reagent Material Safety Data Sheet.

CAUTION: This product contains animal source material. Handle and dispose of this product as if it were potentially infectious.

REAGENT PREPARATION
The reagent is supplied ready to use.

STABILITY AND STORAGE
Prior to use:
When stored refrigerated at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.
Once the Reagent is Opened:
When stored capped at 2-8°C, the reagent is stable until expiry. It is recommended that when the reagent is not in use for prolonged periods of time (eg: overnight) the reagent be capped and stored at 2-8°C.
Indications of Reagent Deterioration:
- Turbidity;
- Reagent Absorbance < 1.4 AU at 340nm (1 cm); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Collection: No special preparation of the patient is required.
Serum: Use non-haemolysed serum. Do NOT use serum preserved with fluoride.
Plasma: Use Sodium heparin or EDTA.
Urine: A 1:20 dilution of urine with ammonia free water is typically required prior to analysis.[4]
Storage: Because of urea's susceptibility to bacterial contamination, it is recommended that all specimens be stored at 2-8°C until analysis. Serum samples are stable for several days at 2-8°C, and for 6 months when frozen (-20°C).[4] Urine samples are stable for 2-3 days at 2-8°C.[5]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- If required, pipettes for accurately dispensing measured volumes.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous Urea standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	340 nm
Secondary Wavelength	405 - 410nm
Assay Type	Fixed Rate
Direction	Decrease
Sample : Reagent Ratio	1:100
eg: Sample Vol	3 µL
Reagent Vol	300 µL
First Read Time	30 seconds
Delay Time	60 Seconds
Last Read Time	90 Seconds
Reagent Blank Limits	Low 1.4 AU
(340nm, 1cm lightpath)	High 2.5 AU
Linearity	0.5 - 40 mmol/L
(refer to linearity section)	(3 - 112 mg/dL)
Analytical Sensitivity	0.01 ΔA/min per mmol/L
(340nm, 1cm lightpath)	3.6 ΔmA/min per mg/dL

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Urea} = \frac{\Delta\text{Abs/min of Unknown}}{\Delta\text{Abs/min of Calibrator}} \times \text{Calibrator Value}$$

$\Delta A/\text{min.} = (A2 - A1)$

Where:
A1 = Absorbance at First Read time
A2 = Absorbance at Last Read time

Example:
Absorbance of Unknown = 0.10 ΔAbs/min
Absorbance of Calibrator = 0.14 ΔAbs/min
Value of Calibrator = 14.3 mmol/L Urea; or 40 mg/dL Urea Nitrogen (See note 3)

$$\text{Urea} = \frac{0.10}{0.14} \times 14.3 = 10.2 \text{ mmol/L}$$

$$\text{Urea} = \frac{0.10}{0.14} \times 40 = 29 \text{ mg/dL}$$

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NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with Urea concentrations greater than 40 mmol/L (112 mg/dL) should be diluted with ammonia free water and reassayed. Multiply results by the dilution factor.
3. Units: Where applicable, values in this insert that are expressed as mg/dL are Urea Nitrogen values.
   mmol/L of Urea x 2.802 = mg/dL of Urea Nitrogen
   mmol/L of Urea x 6.02 = mg/dL of Urea

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.

However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling above the upper limit or below the lower limit of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 522 mg/dL.
   Free Bilirubin: No interference from free bilirubin up to 331 µmol/L (19 mg/dL).
   Conjugated Bilirubin: No interference from conjugated bilirubin up to 310 µmol/L (18 mg/dL).
   Lipaemia: No interference from lipaemia, measured as absorbance at 630nm, up to 1.68 AU.
2. For a more comprehensive review of factors affecting urea assays refer to the publication by Young.[6]

EXPECTED VALUES

Serum[1]	Urea:	2.5-6.4 mmol/L (15-38 mg/dL)
	Urea Nitrogen:	7-18 mg/dL
Urine[4]	Urea:	0.25-0.57 mol/24 Hrs (1.5-3.4 mg/24 Hrs)
	Urea Nitrogen:	7-16 g/24 Hrs

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population it serves.[7]

PERFORMANCE DATA

The following data was obtained using the Infinity Urea Liquid Stable Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.[8]

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.15 / 14.4	18.19 / 51.0
SD (mmol/L / mg/dL)	0.28 / 0.8	0.36 / 1.0
CV (%)	5.3	2.0
Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.15 / 14.4	18.19 / 51.0
SD (mmol/L / mg/dL)	0.41 / 1.1	0.76 / 2.1
CV (%)	8.1	4.2

METHOD COMPARISON

Comparison studies were carried out using a similar commercially available reagent as a reference. Serum and urine samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Serum:	
Number of sample pairs	60
Range of sample results	3.1 - 22.9 mmol/L (9 - 64 mg/dL)
Mean of reference method results	6.9 mmol/L (19 mg/dL)
Mean of Infinity Urea results	6.9 mmol/L (19 mg/dL)
Slope	0.9801
Intercept	0.06 mmol/L (0.2 mg/dL)
Correlation coefficient	0.9936
Urine:	
Number of sample pairs	41
Range of sample results	17.1 - 500 mmol/L (48 - 1398 mg/dL)
Mean of reference method results	280 mmol/L (784 mg/dL)
Mean of Infinity Urea results	261 mmol/L (730 mg/dL)
Slope	0.931
Intercept	0.18 mmol/L (0.5 mg/dL)
Correlation coefficient	0.995

LINEARITY

When run as recommended the assay is linear between 0.5 and 40 mmol/L of Urea (3 and 112 mg/dL Urea Nitrogen).

Linearity on various automated instruments may vary from this value. The user should consult the specific Infinity Urea instrument application.

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.01ΔA/min per mmol/L or 3.6 ΔmA/min per mg/dL (1cm light path, 340nm).

REFERENCES

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3. Tiffany TO, Jansen J.M, Butris CA, Overton JB, Scott CD. Clin Chem 1972; 18:829-40.
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5. Shephard MD, Mezzachi RD. Clin Biochem Revs 1983;4:61-7.
6. Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3:292-301.
7. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
8. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, 1984, NCCLS Publication EP5-T.

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REF

Reorder Information

Catalogue No.	Configuration
TR12421	2 x 125 mL

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