

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

INTENDED USE

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

CLINICAL SIGNIFICANCE¹

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.



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 α -ketoglutarate (α -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

| | <u>Concentration</u> |
|-------------------------|----------------------|
| 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 \pm 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.

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.

SYMBOLS IN PRODUCT LABELLING

| | | | |
|---|------------------------------|---|--|
|  | Authorized Representative |  | Temperature Limitation |
|  | For in vitro diagnostic use |  | Use by/Expiration Date |
|  | Batch code/Lot number |  | CAUTION. CONSULT INSTRUCTIONS FOR USE. |
|  | Catalogue number |  | Manufactured by |
|  | Consult instructions for use | | |

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 μ L |
| Reagent Vol | 300 μ L |
| 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 Δ A/min per mmol/L |
| (340nm, 1cm lightpath) | 3.6 Δ mA/min per mg/dL |

CALCULATIONS

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

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

$$\Delta\text{A/min.} = (\text{A}_2 - \text{A}_1)$$

Where:

- A1 = Absorbance at First Read time
A2 = Absorbance at Last Read time

Example:

| | | |
|--------------------------|---|---|
| Absorbance of Calibrator | = | 0.10 Δ Abs/min |
| Absorbance of unknown | = | 0.14 Δ Abs/min |
| Value of Calibrator | = | 14.3 mmol/L Urea; or 40 mg/dL Urea Nitrogen |
| | | (See note 3) |

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

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

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- 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.
- 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

- 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.

- 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).

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REF

Reorder Information

| Catalogue No. | Configuration |
|---------------|-----------------------|
| TR12421 | 2 x 125 mL |
| TR12498 | 2 x 500 mL |
| 1774-400H | 4 x 100 mL (Hitachi) |
| TL12401 | 8 x 100 mL (ILab 600) |
| TY12401 | 4 x 50 mL (Hitachi) |

** Patented: 7,105,52 - Australia, 5,804,402 - United States, 0817841 - Europe