**INTENDED USE**
This reagent is intended for the in vitro quantitative determination of Creatinine in human serum on automated clinical chemistry analysers.

**CLINICAL SIGNIFICANCE**
Creatinine is a waste product formed in muscle from the high energy storage compound, creatine phosphate. The amount of creatinine produced is fairly constant (unlike urea) and is primarily a function of muscle mass. It is not greatly affected by diet, age, sex or exercise. Creatinine is removed from plasma by glomerular filtration and then excreted in urine without any appreciable resorption by the tubules.

Creatinine is used to assess renal function, however serum creatinine levels do not start to rise until renal function has decreased by at least 50%.

**METHODOLOGY**
Creatinine reacts with alkaline picrate to produce a reddish colour complex (Jaffe reaction). Specificity of the assay has been improved by the introduction of a kinetic method however, the cephalosporin antibiotics are still major interferants.

The red colour formed is directly proportional to the creatinine concentration and is measured spectrophotometrically at 500 nm.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>Picric Acid</td>
<td>10 mmol/L</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>260 mmol/L</td>
</tr>
<tr>
<td>Surfactants</td>
<td>pH 13.0 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

** Assay Procedure**

- Use a clinical chemistry analyser capable of maintaining constant temperature (18-25°C) or for 1 week at 2-8°C.
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 500 and 520nm.
- Analyser specific consumables, eg: sample cups.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous Creatinine standard.

**System Parameters**

- Temperature: 37°C
- Primary Wavelength: 500 nm (500 - 520nm)
- Secondary Wavelength: 550 nm - 600nm
- Assay Type: Fixed Rate
- Direction: Increase
- Sample Volume: 30 µL
- Reagent Volume: 300 µL
- First Read Time: 60 Seconds
- Delay Time: 120 Seconds
- Last Read Time: 180 Seconds
- Reagent Blank Limits: Low 0.0 AU (500nm, 1cm lightpath) High 0.6 AU (500nm, 1cm lightpath)
- Linearity: 0 - 1800 µmol/L (0 - 20 mg/dL)
- Analytical Sensitivity: 0.14 A/min per µmol/L (500nm, 1cm lightpath) 0.012 A/min per mg/dL

**Calculation**

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

\[
\text{Creatinine} = \frac{\Delta \text{Abs/min of Unknown} \times \text{Calibrator Value}}{\Delta \text{Abs/min of Calibrator}}
\]

\[
\Delta \text{Abs / min} = \frac{(A2 - A1)}{2}
\]
Preventative maintenance is performed or a critical component is contingent upon optimum instrument performance and the use of reagents assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments refer to calibration stability. Calibration is required. An aqueous standard or serum based calibrator, with noteS:

3. Unit conversion: µmol/L x 0.0113 = mg/dL.

2. Specimens with creatinine values above 1800 µmol/L should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.

1. The reagent and sample volumes may be altered proportionally to noteS:

Creatinine = \[\frac{0.038}{0.062}\] x 5.0 = 3.1 mg/dL

Creatinine = \[\frac{0.038}{0.062}\] x 440 = 270 µmol/L

Value of calibrator = 440 µmol/L (5.0 mg/dL)

Abs/min of unknown = 0.038

∆Abs/min of calibrator = 0.062

example:

A2 = Absorbance at Last Read time

A1 = Absorbance at First Read time

A2 - A1 = \[\frac{0.038}{0.062}\] x \[\frac{5.0}{440}\] = 3.1 mg/dL

LEVEL I LEVEL II

Within run: SD (µmol/L / mg/dL) 8 / 0.09 13 / 0.15

Between Day: SD (µmol/L / mg/dL) 8 / 0.09 13 / 0.15

Correlation coefficient 0.998

Creatinine = 53 - 97 µmol/L (0.6 - 1.1 mg/dL)

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.

PERFORMANCE DATA

The following data was obtained using the Infinity Creatinine 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 using two levels of commercial control and following the NCCLS EP5-T procedure. The following statistics were obtained.

Number of sample pairs 50

Range of sample results 60 - 1170 µmol/L (0.68-13.20 mg/dL)

Mean of reference method results 210 µmol/L (2.4 mg/dL)

Mean of Infinity Creatinine results 200 µmol/L (2.3 mg/dL)

Slope 0.95

Intercept -4 µmol/L (-0.04 mg/dL)

Correlation coefficient 0.998

LINEARITY

When run as recommended the assay is linear between 0 and 1800 µmol/L (0 - 20 mg/dL). Linearity on automated instruments may vary from the quoted value. It is recommended that the user refer to the appropriate Creatinine instrument application for the instrument specific linearity claim.

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.14 µmol/L/min per µmol/L or approximately 0.0128 Abs/min per mL (1 cm light path, 500nm).

REFERENCES


