Intended Use

The DRI® Creatinine-Detect® Test is intended for the quantitative determination of creatinine in human urine for the detection of urine adulteration by dilution or substitution with non-urine solution.

Summary and Explanation of the Test

A complete urine drug of abuse testing program normally involves specimen collection, initial screening with an immunoassay, followed by a confirmation test, such as gas chromatography/mass spectrometry (GC/MS), for positive samples. Many drug users attempt to evade detection by adulterating their specimen in order to produce false negative results during the initial immunoassay screening. Adulteration methods include dilution with water, substitution with a drug free liquid, addition of readily available household materials (e.g., vinegar, baking soda, liquid drain opener, detergent, etc.) or tampering with certain chemicals (e.g., Urine-Aid, which contains glutaraldehyde or Klear, which contains potassium nitrate).

Several methods have been used to detect urine adulteration. These methods include measuring the temperature, pH, specific gravity and creatinine concentration of the sample. Fresh normal urine should have the following typical characteristics: temperature between 32.5-37.7°C or 90.5-99.8°F, pH within 4.7-7.8, specific gravity within a range of 1.003-1.035 g/mL and creatinine concentration of 80-200 mg/dL. If any of these urine parameters is outside the specified range, there should be reason to believe that the urine sample has been adulterated.

Creatinine is secreted from muscle into urine daily. In the absence of renal disease, rate of creatinine clearance in an individual is relatively constant. Dilution of urine with water or any other non-urine solution can result in a lower creatinine concentration.

DRI Creatinine-Detect Test can be performed on automated clinical chemistry analyzers to measure creatinine concentration. This method is based on the Jaffe reaction, whereby creatinine concentration is determined colorimetrically using alkaline picrate to form a reddish Janovski complex according to the following equation:

\[
\text{Creatinine} + \text{Picric Acid} \xrightarrow{\text{NaOH}} \text{Janovski Complex (Red)}
\]

The color intensity is directly proportional to the creatinine concentration and is measured spectrophotometrically at 505 nm.

Materials Provided

Creatinine-Detect Reagent 1: Contains 500 mL of sodium hydroxide in an aqueous solution.

Creatinine-Detect Reagent 2: Contains 500 mL of picric acid in an aqueous solution.

Calibrators and Controls (sold separately):

Creatinine-Detect Calibrator Kit: Contains 1 x 25 mL of 2.0 mg/dL creatinine and 1 x 25 mL of 20.0 mg/dL creatinine in an aqueous solution.

Results and Data Interpretation

A linear calibration curve is generated to calibrate the assay. The sample creatinine concentration is extrapolated from the calibration curve using the absorbance value of the sample. Most clinical chemistry analyzers have built-in curve-fit software that can calculate the creatinine concentration values automatically with no additional requirement of data manipulation. The 2.0 mg/dL calibrator is used to determine if the urine sample is substituted and the 20.0 mg/dL calibrator is used to determine if the sample is diluted.
Expected Values
Creatinine concentration in normal urine samples range from 80-200 mg/dL. Urine samples with < 20 mg/dL creatinine are considered to be adulterated. Adulteration of urine by substitution of urine sample with non-urine solution will give creatinine concentration < 2 mg/dL.

Limitations
This assay is optimized for the quantitative determination of creatinine in human urine for adulteration purposes only.

Typical Performance Characteristics
The following typical performance data were generated with a Hitachi 717 clinical chemistry analyzer:

Precision
The within-run and total precision was evaluated with three levels of creatinine controls with the following results:

<table>
<thead>
<tr>
<th>Control</th>
<th>Within-run Precision (n=60)</th>
<th>Total Precision (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (mg/dL)</td>
<td>% CV</td>
</tr>
<tr>
<td>1.3</td>
<td>1.2 ± 0.04</td>
<td>3.2</td>
</tr>
<tr>
<td>7.5</td>
<td>7.4 ± 0.10</td>
<td>1.3</td>
</tr>
<tr>
<td>23.0</td>
<td>23.6 ± 0.30</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear from 0.78 mg/dL to 420 mg/dL. Assay linearity was determined by testing serial dilutions of a 600 mg/dL creatinine sample. A correlation of 1.000 was obtained when the observed creatinine concentration of each solution was plotted against its corresponding expected creatinine concentration.

Interference by Endogenous Substances
Interference of endogenous substances in urine was studied. No interference was observed when urine samples were spiked with endogenous substances up to the concentration indicated.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>20 mg/mL</td>
</tr>
<tr>
<td>Galactose</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Glucose</td>
<td>3000 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>300 mg/dL</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>7.5 mg/dL</td>
</tr>
<tr>
<td>Urea</td>
<td>6000 mg/dL</td>
</tr>
</tbody>
</table>

Accuracy and Correlation
Eighty urine samples were tested using the previous by available calibrators, 5 and 20 mg/dL, (x) and 20 calibrators 2.0 and 20.0 mg/dL calibrators (y). Correlation analysis yielded a linear regression equation of y = 0.990x+1 and a correlation coefficient of 1.000.

Bibliography