Creatinine Reagent

INTENDED USE
FOR IN VITRO DIAGNOSTIC USE
Creatinine Reagent is intended for the quantitative determination of creatinine in serum, plasma and urine.

SUMMARY
Serum creatinine is a waste product formed by the spontaneous dehydration of body creatine. Most of the body creatine is found in muscle tissue where it is present as creatine phosphate and serves as a high-energy storage reservoir for conversion to adenosine triphosphate. The rate of creatinine formation is fairly constant with about 2 percent of the body creatine being converted to creatinine every 24 hours.1 Serum creatinine and urea levels are elevated in patients with renal malfunction, especially decreased glomerular filtration. A single, random measurement of serum creatinine may be used as an indicator of impaired kidney function. In the early stages of kidney damage, the rise in the serum urea levels usually precedes the increase in serum creatinine. This advantage is offset by the fact that serum urea levels are affected by factors such as diet, degree of hydration, and protein metabolism. Conversely, serum creatinine levels tend to be constant and unaffected by factors affecting serum urea levels. Thus serum creatinine is a significantly more reliable renal function screening test than serum urea.2 A considerably more sensitive test for measuring glomerular filtration is the creatinine clearance test.1 For this test, a precisely timed urine collection (usually 24 hours) and a blood sample are needed. Urine creatinine levels, as well as other markers of dilution, i.e., specific gravity and appearance, are useful as an adjunct to drug abuse testing in determining external dilution or excessive donor hydration.3,4 Most creatinine assays are based on a modification of the original Jaffe reaction.5 In many of these procedures, the sample is added to an alkaline picrate solution, and the absorbance is read after the reaction has reached end point. Although relatively simple and convenient, the end point assays are somewhat non-specific and subject to interferences by serum metabolites. Improvements in the end point assays have been made by using cation-exchange resins and Lloyd’s reagent to remove the creatinine from the sample.6 Though effective, these procedures are time consuming and require centrifugation or filtration steps. More recently, kinetic assays using the Jaffe reaction have been developed to increase the specificity for creatinine and to reduce the interference by serum metabolites.7

PRINCIPLE OF PROCEDURE
The Creatinine Reagent uses a kinetic modification of the Jaffe reaction.

Creatinine + Picric Acid  \[ \text{Alkaline} \rightarrow \text{red complex} \]

The rate of formation of the red colored complex is measured at 500 nm on the COBAS MIRA® and is proportional to the creatinine concentration in the sample. On COBAS instruments, a time interval for measuring the reaction is chosen to minimize non-creatinine interferences.

REAGENTS

Reagent 1
Sodium Borate 215 mmol/L
pH 13.1

Reagent 2
Picric Acid 52 mmol/L
Sodium Borate 35 mmol/L
pH 8.0
Nonreactive ingredient
Stabilizers
The final reaction mixture contains
Sodium Borate 96 mmol/L
Picric Acid 20 mmol/L
pH 13.0
Nonreactive ingredients
Stabilizers

STORAGE AND STABILITY
Store the reagents at 15-30 °C. The reagents are stable until the expiration date on the label. Keep reagent bottles tightly closed when not in use. Once opened, Reagent 1, Reagent 2, and combined (working) Creatinine Reagent are stable for 21 days when stored in a tightly closed container and 6 hours when exposed to atmosphere. Discard the reagent if it appears turbid or fails to recover stated values for the control sera. Reagent 1 should be clear and colorless; Reagent 2 and the working reagent should be clear and yellow in color.

WARNINGS AND PRECAUTIONS
1. Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety: Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)
2. Results should be interpreted considering all other test results and clinical status of the patient.
3. Do not use washed cuvettes.
4. Avoid contact of reagent with eyes, skin and clothing. Do not pipette reagents by mouth. Do not ingest. Wash hands after use.
5. Corrosive (Sodium hydroxide.) Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately.

SPECIMEN COLLECTION
Either serum, EDTA, lithium or sodium heparin plasma or urine may be used with the appropriate system parameter settings. Sample collected by standard technique. Creatinine in the serum or plasma sample is stable for 24 hours at 2-8°C. Creatinine in the urine sample may be stored at 2-8°C for 4 days. Both samples may be frozen for longer storage.

INTERFERING SUBSTANCES
The following compounds at the indicated levels gave less than 0.1 mg/dL interference as apparent creatinine when added to a normal serum pool.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>25 mg/dL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>10 mg/dL</td>
</tr>
<tr>
<td>Albumin</td>
<td>3 g/dL</td>
</tr>
<tr>
<td>Fructose</td>
<td>200 mg/dL</td>
</tr>
<tr>
<td>Glucose</td>
<td>30 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>10 g/L</td>
</tr>
<tr>
<td>β-hydroxybutyrate</td>
<td>10 mmol/L</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.2 mmol/L</td>
</tr>
<tr>
<td>Uric acid</td>
<td>20 mg/dL</td>
</tr>
</tbody>
</table>

Cephalosporin antibiotic interferences with Jaffe methods for creatinine have been described.8 When tested at the concentrations indicated below, the following cephalosporin antibiotics spiked into serum containing a level of 1.0 mg/dL creatinine were found to give the amount of interference indicated:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefaclor</td>
<td>0.7 mg/dL</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>5.3 mg/dL</td>
</tr>
<tr>
<td>Cephaproleine</td>
<td>0.2 mg/dL</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0.2 mg/dL</td>
</tr>
</tbody>
</table>

Less than 10% interference was demonstrated from up to 600 mg/dL hemoglobin F, young gives a list of drugs and other substances that interfere with the determination of creatinine.

PROCEDURE

Materials provided

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>R85559</td>
<td>1 x 100 mL</td>
</tr>
<tr>
<td>R85559V1</td>
<td>2 x 100 mL</td>
</tr>
<tr>
<td>R85546V1</td>
<td>10 x 3 mL</td>
</tr>
<tr>
<td>R85546V2</td>
<td>10 x 3 mL</td>
</tr>
</tbody>
</table>

Materials required but not provided

1. COBAS MIRA® chemistry analyzer.
2. Multi-Analyte Serum Calibrator (R60010)

TEST PROCEDURE

Pour sufficient working reagent into the proper position on the reagent rack and place on the instrument. R85546 contains vials that are COBAS MIRA® system rack compatible. See appropriate operator’s manual for further details on system programming and operation.

CALIBRATION

Use Multi-Analyte Serum Calibrator (R60010) for serum samples. Consult the package insert for this product for instructions on use. Calibrate in accordance with the instrument manufacturer’s specifications. Calibration stability is dependent upon the instrument performance and the proper storage of the reagents. Re-calibration is recommended at any time, should one of the following occur.

1. Change in the reagent lot number.
2. Preventative maintenance is performed on the analyzer.
3. A critical element of the analyzer is replaced.
4. Control material results have shifted or are out of range and the use of a freshly reconstituted vial of control does not correct the situation.

Each laboratory should establish its own procedures for corrective action if calibration is not acceptable.

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QUALITY CONTROL
Controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Assayed Control Serum Level 1 (R83082) and Assayed Control Serum Level 2 (R83083) are recommended for this purpose. Each laboratory should establish its own control schedule.

CALCULATIONS
COBAS chemistry instruments calculate a factor using the following formula:

\[
F = \frac{C_{STD1}}{C_{STD2}} \times \frac{\Delta A_{STD1} - \Delta A_{RB}}{\Delta A_{STD2} - \Delta A_{RB}}
\]

then:

\[
\text{Concentration of the sample (mg/dL)} = (\Delta A_{sample} - \Delta A_{RB}) \times F
\]

where:

\[
C = \text{Concentration in mg/dL}
\]

\[
STD1 = \text{Standard One (first replicate)}
\]

\[
STD2 = \text{Standard One (second replicate)}
\]

\[
\Delta A = \text{Absorbance Change}
\]

\[
RB = \text{Reagent Blank}
\]

\[
F = \text{Factor}
\]

CREATININE CLEARANCE
The creatinine clearance is calculated using the following formula:

\[
\text{Creatinine clearance (mL/min)} = \frac{U \times V \times 1.73}{P \times A}
\]

where:

\[
U = \text{Concentration of creatinine in the urine}
\]

\[
P = \text{Concentration of creatinine in the serum or plasma}
\]

\[
V = \text{Volume of urine in ml/min}
\]

\[
A = \text{Body surface area in square meters}
\]

\[
1.73 = \text{Average body surface in square meters}
\]

The patient's body surface may be calculated using the following formula or obtained more conveniently from an available nomogram.

\[
\log A = (0.425 \log W) + (0.725 \log H) - 2.144
\]

where:

\[
A = \text{The body surface area in square meters}
\]

\[
W = \text{The weight of the patient in kg}
\]

\[
H = \text{The height of the patient in cm}
\]

PROCEDURAL LIMITATIONS
Samples with creatinine values greater than 30 mg/dL for the serum application or 200 mg/dL for the urine application should be diluted with 0.9% NaCl or deionized water and re-assayed. Correct the results for the dilution factor.

Cobas MIRA® systems with automatic post-dilution extend the serum and plasma assay linearity to 60 mg/dL.

A result obtained with this reagent should serve only as a diagnostic aid and is not to be interpreted as diagnostic in and of itself. A result obtained with this reagent should serve only as a diagnostic aid and is not to be interpreted as diagnostic in and of itself. A reagent obtained with this reagent should serve only as a diagnostic aid and is not to be interpreted as diagnostic in and of itself.

The following table lists the data obtained in a comparison of urine samples with the reagent for Creatinine (y) with a similar creatinine reagent (x) run on a COBAS MIRA®.

<table>
<thead>
<tr>
<th>Sample Pairs</th>
<th>Mean (mg/dL)</th>
<th>CV%</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>72.7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Level 2</td>
<td>136.3</td>
<td>20</td>
</tr>
</tbody>
</table>

Urine specimens with low and high concentrations of creatinine on a COBAS MIRA showed the following performance:

<table>
<thead>
<tr>
<th>Sample Pairs</th>
<th>Mean (mg/dL)</th>
<th>CV%</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>4.0</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Level 2</td>
<td>9.4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

EXPECTED VALUE
A study was conducted on a total of 100 samples from normal blood donors (52 females and 48 males) using the Creatinine Reagent. The expected values were found to be 0.7 - 1.4 mg/dL for the female donors and 0.9 - 1.5 mg/dL for the male donors. In a creatinine clearance study consisting of 18 donors, the expected values were found to be 69 - 149 ml/min.

A study was conducted on a total of 50 single, random urine samples from normal donors using the Creatinine Reagent. The range of values was found to be 15.8 - 260.8 mg/dL with a mean of 64.8 mg/dL and a SD of 30 mg/dL. Five values were below 30 mg/dL and one was below 20 mg/dL.

It is recommended that each laboratory establish their normal range.

REFERENCES
2. Henry JB. Clinical Diagnosis and Management by Laboratory Methods, 16th ed. WB Saunders, Philadelphia, PA, 1974; 263.


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