Intended Use
For the quantitative enzymatic determination of creatinine in serum and urine on the Mindray BS-480. For In Vitro Diagnostic Use Only.

Principle
Creatinine is a catabolic product of creatine, which is used in skeletal muscle contraction. The daily production of creatine, and subsequently creatinine, depends on muscle mass, which fluctuates very little. Creatinine is excreted entirely by the kidneys and therefore is directly proportional to renal excretory function. Thus with normal renal excretory function, the serum creatinine level should remain constant and normal. Only renal disorders, such as glomerulonephritis, pyelonephritis, acute tubular necrosis, and urinary obstruction, will cause an abnormal elevation in creatinine.1

The current method employs a two reagent system which eliminates interference by endogenous creatine and ascorbic acid.

Creatinine amidohydrolase
Creatinine + H₂O → Creatine

Creatine amidinohydrolase
Creatine + H₂O → Sarcosine + Urea

Sarcosine oxidase
Sarcosine + H₂O + O₂ → Glycine + HCHO + H₂O₂

Peroxidase
2H₂O₂ + 4-aminoantipyrine + *ESPMT → Quinoneimine Dye + 4 H₂O

*ESPMT: N-ethyl-N-sulphonyl-m-toluidine

Reagents
Creatinine Enzyme Buffer Reagent (R1): Good Buffer (pH 7.4) 25 mmol/L, Creatine amidohydrolase > 25 KU/L, Sarcosine oxidase > 7 KU/L, Ascorbate oxidase > 4 KU/L, ESPMT 140 mg/L

Creatinine Enzyme Color Reagent (R2): Good Buffer (pH 7.3) 100 mmol/L, Creatine amidohydrolase > 250 KU/L, Peroxidase > 5 KU/L, 4-aminoantipyrine 600 mg/L, ESPMT

Reagent Preparation
Reagents are provided as ready to use liquids.

Reagent Storage and Stability
Reagents are stable until expiration dates found on their labels when stored at 2-8°C. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Precautions and Hazards
Hazards:
R1 and R2:
Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
 Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture.

Specimen Collection and Storage
1. Serum: Remove specimen from clot promptly to prevent hemolysis.
2. Do not use fluoride or ammonium heparinate to collect sample.²

Sample Stability: Creatinine values have a reported stability of one day at 2-8°C, and several months when frozen (-20°C) and protected from evaporation and contamination. Store urine at 2-8°C.²

Interferences
No interference was observed by ascorbic acid up to 200 mg/dL, hemoglobin up to 500 mg/dL, bilirubin-conjugate up to 32 mg/dL, and bilirubin-free up to 40 mg/dL. An extensive list of drugs or other agents interfering with creatinine methodologies has been reported by Young et al³.

Materials Provided
1. Creatinine R1 Reagent
2. Creatinine R2 Reagent
Creatinine (Enzymatic)
Reagent Set

Materials Required but not Provided
1. Mindray BS-480
2. BS-480 Operation Manual
3. Chemistry control, catalog number CHEQ480
4. Chemistry Calibrator, catalog number CHEC480

Calibration
If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Quality Control
Two (2) levels of control material with known Creatinine levels determined by this method, should be analyzed each day of testing.

Expected Values
Normal Range: Male (serum): 0.9 - 1.5 mg/dL
Male (urine): 1000 - 2000 mg/24hrs.
Female (serum): 0.7 - 1.4 mg/dL
Female (urine): 600 - 1500 mg/24hrs.

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories and local populations.

Performance
1. Assay Range: 0.01-30.00 mg/dL. Samples exceeding this value should be diluted 2-fold with deionized water, the assay repeated and results multiplied by 2.
2. Correlation: A study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

<table>
<thead>
<tr>
<th>Method</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
</tr>
<tr>
<td>Mean Creatinine (mg/dL)</td>
<td>3.945</td>
</tr>
<tr>
<td>Range (mg/dL)</td>
<td>0.49-22.73</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>5.725</td>
</tr>
<tr>
<td>Regression Analysis</td>
<td>y = 1.046x + 0.036</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9994</td>
</tr>
</tbody>
</table>

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>LOW</th>
<th>MID</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>1.262</td>
<td>4.141</td>
<td>24.984</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.011</td>
<td>0.019</td>
<td>0.064</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>0.9%</td>
<td>0.5%</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>LOW</th>
<th>MID</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mean</td>
<td>1.286</td>
<td>4.212</td>
<td>25.161</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.021</td>
<td>0.055</td>
<td>0.359</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>1.6%</td>
<td>1.3%</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

4. Sensitivity: 2SD limit of detection (95% Conf) = 0.01 mg/dL

Data obtained on Hitachi 717

5. Urine specimens (n = 37) were assayed by this method and by another commercial method. Statistical analysis revealed a correlation coefficient (r) of 0.9854, with a regression equation of y = 1.0545x + 0.3607.

References
5. Manufacturer’s Laboratory Data
CHEMISTRY PARAMETERS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Chem:</td>
<td>CRET-enz</td>
</tr>
<tr>
<td>Chemistry:</td>
<td>Creatinine (Enzymatic)</td>
</tr>
<tr>
<td>Reaction Type:</td>
<td>End Point</td>
</tr>
<tr>
<td>Pri Wave:</td>
<td>546</td>
</tr>
<tr>
<td>Unit:</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Blank Time:</td>
<td>47 49</td>
</tr>
<tr>
<td>Sample Vol. Aspirated Diluent</td>
<td>2.0 ul --- ul --- ul</td>
</tr>
<tr>
<td>Standard:</td>
<td>2.0 ul</td>
</tr>
<tr>
<td>Decreased:</td>
<td>--- ul</td>
</tr>
<tr>
<td>Increased:</td>
<td>--- ul</td>
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<tr>
<td>Sample Blank</td>
<td>☐</td>
</tr>
<tr>
<td>Auto Rerun</td>
<td>☑</td>
</tr>
<tr>
<td>Reaction Time:</td>
<td>80 82</td>
</tr>
<tr>
<td>Reaction Vol. Diluent:</td>
<td>R1: 120 ul --- ul</td>
</tr>
<tr>
<td>R2: 40 ul --- ul</td>
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</tr>
<tr>
<td>R3: --- ul --- ul</td>
<td></td>
</tr>
<tr>
<td>R4: --- ul --- ul</td>
<td></td>
</tr>
</tbody>
</table>

**Slope/Offset Adjustment**

- **Slope**: 1
- **Offset**: 0

**Linearity Range**

- Standard: 0.01 30
- Decreased: __ __
- Increased: __ __

**Substrate Depletion**

- Mixed Blank Abs: __ __

**Uncapping Time**

- __ __

**Reagent Alarm Limit**

- __ __

**Twin Chemistry**

- ☐ Enzyme Linear Extension
- ☐ Prozone Check
- ☐ Rate Check
- ☐ Antigen Addition

**Chemistry Parameters**

- Q1: __
- Q2: __
- Q3: __
- Q4: __

- PC: ABS:
CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: *  Lot No.: *
Exp Date: *

Carousel Pos
Sample Carousel 1 *
Sample Carousel 2
Sample Carousel 3

Reagent/Calibration

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Pos</th>
<th>Lot No.</th>
<th>Exp Date</th>
<th>Chem</th>
<th>Conc</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>W</td>
<td>*</td>
<td>*</td>
<td>CRET-enz</td>
<td>0</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Chemistry Calibrator</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>CRET-enz</td>
<td>*</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Calibration Setup

Chem: CRET-enz

Calibration Settings

Math Model: Two-Point Linear
Factor: Replicates: 2

Acceptance Limits

Cal Time: *  Hour
Slope Diff: ---  SD: ---
Sensitivity: ---  Repeatability: ---
Deter Coeff: ---

Auto Calib.
☐ Bottle Changed  ☐ Lot Changed  ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF  CEE480  Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

Symbol Key

- Use by (YYYY-MM-DD)
- Lot and batch code
- Catalog number
- Manufacturer
- Consult instructions for use
- In vitro diagnostic medical device