Name **Creatinine**  
(Sigma) Kit #555-A

**Principle**
Most methods used for creatinine determination are based upon the Jaffe reaction, in which creatinine forms a characteristic yellow-orange color when treated with alkaline picrate. The color produced, however, is not specific for creatinine and interfering substances can alter test results, especially proteins. Recently several methods which improve specificity have been developed.

Creatinine reacts with picric acid under alkaline conditions to form a yellow-orange complex. The color is derived from creatinine as well as certain other non-specific substances. Upon the addition of acid, the color contributed by creatinine is destroyed, while that produced by non-specific substances remains. The difference in color intensity measured at 500nm before and after acidification is proportional to the creatinine concentration.

**Clinical Significance**
When there is impaired formation or elimination of urine, there is an **increase** in several compounds present in the blood plasma. These compounds are relatively small nitrogen-containing molecules that are collectively called the “non-protein nitrogen constituents” of plasma or serum. The compounds whose serum concentrations are of great significance in kidney disease are creatinine and urea and, to a lesser degree, uric acid. Circulating levels of creatinine are used primarily as an index of renal function. **High** plasma creatinine concentrations are encountered in nephritis and renal obstruction, reflecting the degree of impairment.

**Normal Values**

<table>
<thead>
<tr>
<th></th>
<th>Serum: Men</th>
<th>0.9-1.4 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>0.8-1.2 mg/dl</td>
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<tr>
<td>Urine:</td>
<td>Men</td>
<td>1.1-2.8 g/24 hr</td>
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<tr>
<td></td>
<td>Women</td>
<td>0.9-1.6 g/24 hr</td>
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<tr>
<td>Clearance:</td>
<td>Men</td>
<td>105 ± 20 mL/min</td>
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<tr>
<td></td>
<td>Women</td>
<td>95 ± 20 mL/min</td>
</tr>
</tbody>
</table>

**Acceptable Student Result Range**
Patient samples should be within normal values. Abnormal values should be brought to the attention of an instructor. Controls must be within ± 2 SD.

**Time Frame**
35-45 minutes
Specimen
Either serum or plasma may be used and specimens may be stored in the refrigerator at 0-5°C for 24 hours or for several months frozen. Urine samples containing thymol or preserved with toluene are stable for 24 hours at room temperature or several days in the refrigerator at 0-5°C.

Samples containing bromsulfophthalein or phenolsulfonephthalein may not be used. Bilirubin causes positive interference in this procedure.

Reagents
For In Vitro Diagnostic Use
1. **Sodium Hydroxide Solution:** Contains sodium hydroxide, 1.0 N  
   **Danger:** Corrosive. Avoid contact with skin, eyes or clothing.
2. **Creatinine Color Reagent:** Contains picric acid, 0.6%, sodium borate, and surfactant. If reagent becomes cold, turbidity may develop. Warm reagent to clear. Mix well before use.  
   **Danger:** Poison. Avoid contact with skin. Wipe up any spillage thoroughly.
3. **Acid Reagent:** Contains mixture of sulfuric and acetic acid.  
   **Danger:** Corrosive. Do not pipet by mouth. Avoid getting in mouth, eyes, or on skin.
4. **Creatinine Standard Solution:** Contains creatinine, 3.0 mg/dl in dilute acetic acid. Also a 15 mg/dl standard.

Store creatinine standards in refrigerator at 0.5°C. All other reagents should be stored at room temperature.

Preparation
1. **Alkaline Picrate Solution:** Prepare by mixing 5 volumes of creatinine color reagent with 1 volume of sodium hydroxide solution. Mixture is stable for at least one week stored in the dark at room temperature. It will darken with time, but this does not affect its usefulness.
2. **Sodium Hydroxide Solution:** **Danger:** Corrosive. Avoid contact with skin, eyes or clothing.
3. **Creatinine Color Reagent:** **Danger:** Poison. Avoid contact with skin. Wipe up spills carefully.
4. **Acid Reagent:** **Danger:** Corrosive. Do not pipet by mouth. Avoid getting in mouth, eyes, or on skin.

Materials Required But Not Provided
1. Spectrophotometer capable of measuring 0-2 O.D. units at a wavelength of 500 nm.
2. Cuvets
3. 5 ml serological pipets
4. 1 ml pipets
5. 0.1 ml pipets

**Standardization**

Standardization may be accomplished by means of a prepared calibration curve or by the simultaneous assay of a standard of known creatinine concentration with each run.

(In our lab we will run a standard with each batch of controls and specimens.)

**Procedure**

1. Label cuvets for blank, standard, controls, and tests.
2. Add 0.3 ml specimen to each tube. Use water for the blank.
3. Add 3.0 ml alkaline picrate solution to each tube. Mix and allow to stand at room temperature for at least 10 minutes but **no longer** than 15 minutes.
4. Read initial absorbance of standard and tests, using blank to zero at a setting of 500nm.
5. To all tubes, add exactly 0.1 ml acid reagent. Mix immediately and well. Allow to stand at room temperature for 5 minutes. **NOTE:** A precipitate may form upon the addition of the acid reagent, but it will redissolve after mixing.
6. Read final absorbance of standard and tests using blank to zero at the same wavelength.

**Results**

The creatinine concentration of the samples may be calculated according to the following formula:

**Quality Control**

Two levels of assayed control, ie. Monitrol I and Monitrol II

**References**

Product Insert, *Sigma Creatinine*, Sigma Chemical Company,