

Creatinine

1.0 INTENDED USE

This reagent is intended for the quantitative determination of creatinine in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Jaffe (10.1) first described the reaction of Creatinine with an alkaline picrate solution to form a red color in 1886. Because of its simplicity, precision, and low cost, the Jaffe reaction has been used extensively for the colorimetric determination of Creatinine. The Biotron method is a modification of the Jaffe method using a kinetic measurement of the color formation.

2.2 TEST PRINCIPLE

Creatinine reacts with picric acid in an alkaline medium to produce a red colored complex.

Creatinine + Alkaline Picrate ----> Creatinine-picric acid complex

The rate of formation of this complex, in a selected time interval, is directly proportional to the concentration of creatinine in the sample.

2.3 CLINICAL SIGNIFICANCE (10.4)

Creatinine is not actively reabsorbed from the renal tubules and is excreted via the urine. In normal individuals, the level of creatinine in serum remains low and constant. When the glomerular filtration rate decreases, as in cases of renal insufficiency, the level of serum creatinine will elevate. Since the serum creatinine is less affected by diet and hydration than is the level of urea nitrogen, creatinine is believed to be of greater clinical specificity. At the onset of renal insufficiency, the serum creatinine increases more slowly following recovery of renal function.

Since the creatinine in serum is derived from the creatinine of muscles, increased serum creatinine levels are seen in muscle-wasting disorders such as muscular dystrophy. Increased levels are also found after muscle trauma such as can occur following strenuous exercise. In general, serum creatinine levels directly correlate with muscle mass, i.e., a large muscle mass being reflected in higher serum creatinine levels.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Plasma prepared with lithium heparin may be used.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-200 µl. Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Serum or plasma samples may be stored for 7 days at 2-8°C. Samples may be stored indefinitely at -20°C. (10.2)

Frozen samples should be thawed at room temperature, and mixed completely before analysis. Thawed samples should not be refrozen.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS

(2 X 125 ml)

Reagents necessary for the determination of creatinine are included in the kit.

4.1 REAGENT

Creatinine Reagent contains: picric acid 22 mmol/L

Creatinine Base Reagent contains: sodium hydroxide 450 mmol/L

4.1.1 Standard/Control/Calibrator

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

The working reagent is prepared by mixing equal volumes of creatinine reagent and creatinine base reagent. Mix well before using. Record the date and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

When stored at 18-26°C unopened reagents are stable until the expiration date printed on the label. The working creatinine reagent is stable at 18-26°C for 10 days.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer capable of reading absorbance at 510 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 510 nm.

4.5.3 Test tubes capable of holding 3 ml.

4.5.4 Pipettes capable of delivering 2 ml and 200 µl.

4.5.5 Deionized or distilled water for preparing the reagent blank.

4.5.6 Timer for a 1 minute intervals.

4.5.7 Calibrator .

4.5.8 Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

| | |
|-------------------------|-------------------|
| Wavelength | 510 nm |
| Temperature | 18-26° C or 37° C |
| Pathlength | 1.0 cm |
| Mode | kinetic |
| Reaction time | 1.3 - 2.3 minutes |
| Sample volume | 200 µl |
| Reagent volume | 2.0 ml |
| Total volume | 2.2 ml |
| Sample to reagent ratio | 1/10 |

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 510 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The creatinine assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

- 5.4.1 Prepare the required volume of Creatinine working reagent (see 4.3 Reagent Preparation Section)
- 5.4.2 Into separate test tubes pipette 200 µl of distilled water, calibrator or serum to be assayed.
- 5.4.3 Add 2.0 ml of working reagent and incubate for 20 seconds.
- 5.4.4 Record the absorbance of the calibrator at 510 nm at 20 seconds (As1) and at 80 or 140 seconds (As2). Also record the absorbance of each serum at 510 nm at 20 seconds (A1) and at 80 or 140 seconds (A2).

5.5 CALCULATION AND RESULTS

$$\text{Creatinine (mg/dl)} = \frac{A2 - A1}{As2 - As1} \times \text{concentration of calibrator}$$

A1 = initial absorbance of unknown
A2 = final absorbance of unknown
As1 = initial absorbance of calibrator
As2 = final absorbance of calibrator

Example:

$$\text{Creatinine} = \frac{0.192 - 0.172}{0.180 - 0.057} \times 6.0 \text{ mg/dl} = 0.98 \text{ mg/dl}$$

with A1 = 0.172, A2 = 0.192 and As1 = 0.057, As2 = 0.180, concentration of calibrator = 6.0 mg/dl

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.3)

The range of expected values is: 0.7 - 1.4 mg/dl

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.6)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

Icteric sera can give low values for creatinine. Studies have shown that creatinine values are depressed when bilirubin is 10 mg/dl or higher.

For a list of drugs which may affect creatinine, see Young (10.3.)

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The creatinine assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 15 replicates of 2 control sera were run.

Mean (mg/dl) SD (mg/dl) CV (%)

| | | |
|------|--------|------|
| 1.88 | ± 0.05 | 2.88 |
| 10.1 | ± 0.08 | 0.76 |

Between-Run

In this study, 5 runs were made, each run consisting of 5 replicates of 2 control sera.

| <u>Mean (mg/dl)</u> | <u>SD (mg/dl)</u> | <u>CV (%)</u> |
|---------------------|-------------------|---------------|
| 1.91 | ± 0.03 | 1.70 |
| 10.3 | ± 0.07 | 0.70 |

9.2 CORRELATION

A correlation study was done on the Technicon RA-500 system at 37° C comparing this method and a similar creatinine method. The samples range between 1.1 mg/dl and 16.6 mg/dl.

| Number of Samples | Regression Equation <u>y=Biotron, x=Comparative</u> | Correlation <u>Coefficient</u> |
|----------------------|--|-----------------------------------|
| 30 | y = 1.003 x - 0.0432 | 0.999 |

9.3 LINEARITY

This procedure is linear through 12 mg/dl beyond which the specimen should be diluted 1 to 1 with 0.9% saline. Reassay the specimen and multiply the results by 2.

10.0 REFERENCES

- 10.1 Jaffe, M., Hoppe Selyer's Z. Physiol. Chem. 10,391-400 (1886)
- 10.2 Kaplan, L., Pesce, A., Clinical Chemistry Theory, C.V. Mosby Co., Princeton, NJ (1984)
- 10.3 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
- 10.4 Clinical Diagnosis, 15th ed., I. Davidsohn and J.B. Henry, Eds., W.B. Saunders, Philadelphia, PA, 1974, p.594.
- 10.5 Kaplan, A., Szabo, L.L., Clinical Chemistry: Interpretations and Techniques, 2nd ed., Lea and Febiger, Philadelphia, PA 1983.
- 10.6 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704