CREATININE(Jaffe Kinetic)

INTENDED USE
Bioline Creatinine reagent is used for the quantitative determination of creatinine in serum.

CLINICAL SIGNIFICANCE
Creatinine, an anhydride of creatine,is a waste product formed by the spontaneous dehydration of kidneys. Most of the creatinine is found in muscle tissue where it is present as creatine phosphate and serves as a high energy storage reservoir for conversion to ATP. In-dependent of diet, serum creatinine concentrations depends almost entirely upon its excretion rate by the kidneys. For this reason, its elevation is highly specific for kidney diseases.

METHOD AND PRINCIPLE
The assay of creatinine has been based on the reaction of creatinine with alkaline picrate as described by Jaffe. Further modifications have developed the Jaffe reaction into a kinetic assay that is fast, simple and avoids interferences.

Creatinine+Sodium Picrate $\xrightarrow{\text{Alkaline pH}}$ Creatinine–Picrate complex
(yellow-orange)

Creatinine reacts with picric acid in alkaline conditions to form a color complex which absorbs at 510 nm. The rate of formation of color is proportional to the creatinine concentration in the sample.

REAGENT COMPOSITION
1. Creatinine Picric Acid Reagent: A solution containing 10 mm picric acid.
2. Creatinine Buffer Reagent: A solution containing 240 mM, sodium hydroxide and EDTA.
3. Creatinine standard (2mg/dl): A solution containing creatinine in hydrochloric acid with preservative.

WARNING AND PRECAUTIONS
1. This reagent is for "in vitro" diagnostic use only.
2. Creatinine Picric Acid Reagent is a strong oxidizing agent. Avoid contact with skin. WIPE ANY SPILLAGE, SINCE PICRIC ACID IS EXPLOSIVE.
3. Creatinine buffer reagent is an alkali. Avoid ingestion and contact.

REAGENT PREPARATION
Combine equal volumes of Creatinine Picric Acid Reagent (R2) and Creatinine Buffer Reagent (R1) and, mix well.

REAGENT STORAGE AND STABILITY
1. Both reagents are stored at room temperature (18 - 25°C).
2. Combined (working) reagent is stable for up to one week.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. The reagent fails to meet linearity claims or fails to recover control values in the stated range.

SPECIMEN COLLECTION AND STORAGE
1. Serum is recommended.
2. Creatinine in serum is stable for 24 hours at refrigerated temperatures (2 - 8°C) and several months when frozen(at -20°C) and protected from evaporation and contamination.
3. 24-hour urine specimens must be preserved with 15 grams of boric acid.

INTERFERENCES
A number of substances affect the accuracy of creatinine determination. see Young et al for a comprehensive list.
Albumin at a concentration of 10.0 gm/dl contributes 0.2 mg/dl to the creatinine value, moderate hemolysis (0.2 gm/dl Hb), grossly icteric and lipemic samples will give elevated results.
Acetoacetate above 10mg/dl will interfere with the results.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength 510nm (500-520) nm

Temperature 37ºC

	Standard	Sample
Reagent R1	500 µL	500 µL
Reagent R2	500 µL	500 µL
Standard	100 µL	-
Sample	-	100 µL

Mix and read the optical density (OD1) 30 seconds after the sample or standard addition. At exactly 120 seconds of OD1, take second reading (OD2).

CALCULATIONS
The Creatinine value of the unknown is determined by comparing its absorbance change with that of a known standard.

$$\frac{\Delta Abs.(Unknown)}{\Delta Abs.(Standard)} \times Conc\ of\ Standard = mg/dl(Creatinine)$$

Where:
Δ Abs. =Absorbance change between readings (A_2-A_1)

SAMPLE CALCULATION
If :Abs Δ. (Unknown)= 0.020
Abs Δ. (Standard)=0.050
Conc.of Standard=2mg/dl
Then
$\frac{0.020}{0.050} \times 2 = 0.8$ mg/dl creatinine

CALIBRATION
The procedures are calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results.

QUALITY CONTROL
The integrity of the reaction should be monitored by use of normal and abnormal control sera with known creatinine values.

EXPECTED VALUES

Serum:	Male	0.9-1.5mg/dl
	Female	0.7-1.37mg/dl

PERFORMANCE CHARACTERISTICS
1. Linearity: 15 mg/dl. For samples above 15mg/dl, dilute the sample with saline/DW and rerun. Multiply the obtained result with dilution factor to get correct Creatinine value.
2. Comparison :A study performed between this procedure and a similar kinetic procedure yielded a correlation coefficient of 0.99 with a regression equation of y = 0.96x + 0.06. Serum and control samples used in the study had creatinine values ranging from 0.9 to 8.3 mg/dl.

3. Precision:

Mean mg/dl	S.D.	WithinRun C.V.%
1.9	0.05	2.6
8.2	0.6	4.3

Mean mg/dl	S.D.	Run to Run C.V.%
2.0	0.2	10.0
8.2	0.4	4.6

General Technical Parameters

Mode	**Two Point / Fixed Time**
Wavelength (Filter)	**505 nm**
Reaction Direction	**Increasing**
Sample Vol.	**100 µL**
Reagent Vol.	**1000 µL**
Delay Time / Lag Time	**30 Seconds**
Interval Time	**120 sec**
No of Reading	**1**
Measuring Time / Read time	**120 Seconds**
Calibration Method	**1- Point**
Standard (Conc.)	**2.0 mg/dL**
Linearity	**15 mg/dL**
Decimal Places	**2**
Temp.	**37 °C**
Unit	**mg/dL**
Ref. Low (Male/Female)	**0.9 / 0.7 mg/dL**
Ref. High (Male/Female)	**1.5 / 1.37 mg/dL**

REFERENCES

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