

CREATININE

END POINT & KINETIC

Last update 04/2014

Jaffe's modified method (End Point & Kinetic Method)

(ALKALINE PICRATE METHOD)

Reagent kit for quantitative estimation of CREATININE in serum or urine.

BACKGROUND & SYNOPSIS:

Serum Creatinine determination is mainly used for the diagnosis of renal disease. Creatinine is an endogenous NPN (Non Protein Nitrogen) waste product of the body excreted through kidneys. Creatinine, after filtration in the glomerulus, is not reabsorbed in the tubules and hence urine creatinine measures glomerular filtration rate (GFR). Urine creatinine determination is usually carried out as a part of creatinine clearance test.

Creatinine kit is based on Jaffe's Kinetic method. A drawback of Jaffe's endpoint reaction is the interference due to non-specific substances such as proteins, ascorbic acid and ketoacids. Various modifications are aimed at either eliminating or minimizing this interference. In the kinetic method, a delay, before the picrate creatinine complex formation is monitored, minimizes interference from the fast reacting substances such as ketoacids and hence subsequent measurements upto 120 seconds, largely refers to true creatinine values only. Other advantages of the kinetic reaction includes no deproteinization, rapid method and low sample volume.

PRINCIPLE:

Creatinine reacts with alkaline picrate to produce a red colored complex; the rate of red colored complex formation is directly proportional to the Creatinine concentration.

DIAGNOSTIC SIGNIFICANCE:

Creatinine is a waste product formed in muscle from the high energy storage compound, creatine phosphate. The amount of Creatinine produced is fairly constant and is primarily a function of muscle mass.

Creatinine is removed from plasma by glomerular filtration and then excreted in urine without any appreciable resorption by the tubules.

Creatinine is used to assess renal function, however, serum creatinine levels do not start to rise until renal function has decreased by at least 50%. Congestive heart failure, shocks and mechanical obstruction of urinary tract may also contribute to an elevated level of serum creatinine. An elevated serum creatinine level due to obstruction may rapidly fall when the obstruction is removed by surgery. Many times serum urea/creatinine ratio is used for assessment of kidney function and differential diagnosis. Creatinine clearance test is carried out for assessment of kidney function only.

PRESENTATION:

No. of Bottles

	No. of Bottles
	2 x 100 ml
• 1 CREATININE (Picrate Reagent)	1
• 2 CREATININE (Alkali Reagent)	1
• 3 CREATININE (Acid Reagent)	1
• CREATININE STANDARD (2 mg/dL.)	1

STORE AT 2-8°C
(PROVIDED SEPARATELY)

PRECAUTION:

ENZOPAK Creatinine is for *IN-VITRO* diagnostic use only. Avoid contact of the reagents with skin, eyes and clothes. Use of automated pipetting devices.

WORKING REAGENT PREPARATION:

Prepare working reagent by combining one volume of 1 CREATININE with one volume of 2 CREATININE.

REAGENT STORAGE AND STABILITY:

All the reagents included in the kit are stable at room temperature until the expiry date printed on the label.

SPECIMEN COLLECTION:

Fresh, clear serum with no hemolysis is the specimen of choice. Plasma prepared using heparin as an anticoagulant may also be used.

GENERAL PARAMETERS FOR KINETIC METHOD:

• Type of Reaction	: Fixed Time/Two Point/ Initial Rate
• Wavelength	: 510 nm.
• Flowcell/Cuvette Temperature	: 30°C
• Delay Time	: 20 seconds
• Interval	: 60 seconds
• Measuring Time	: 80 seconds
• No. of readings	: 2
• Standard/Sample Volume	: 100 µl (0.1 ml).
• Standard Concentration	: 2 mg/dL
• Working Reagent Volume	: 1.0 ml.
• Light Path	: 1.0 cm.
• Zero setting with	: Distilled Water.

Note: For instruments using cuvette capacity of 2.5 ml, use sample and standard volume 200 µl (0.2 ml) and working reagent 2.5 ml.

PROCEDURE:

PIPETTE INTO TEST TUBE	STANDARD (ST)	TEST (TS)
WORKING REAGENT (ml)	1.0	1.0
STANDARD (ml)	0.1	-
SAMPLE (ml)	-	0.1

Mix and aspirate. Record the absorbance of Standard (ST) and Test (TS) at 20 seconds (ST₁, TS₁) and again at 80 seconds (ST₂, TS₂) at 510 nm, against distilled water.

TEST RESULTS:

$$\text{Creatinine concentration} = \frac{(TS_2 - TS_1)}{(ST_2 - ST_1)} \times 2$$

To convert (mg/dL) to micromol/liter, use the following equation; micromol/liter = (mg/dL) x 88.5

GENERAL PARAMETERS FOR END POINT METHOD

• Type of Reaction	: Two Step End Point.
• Wavelength	: 510 nm.
• Flowcell Temperature	: 30°C / 37°C.
• Std. Concentration	: 2 mg/dl.
• Sample Volume	: 100 µL (0.1ml).
• Reagent Volume	: 1 ml.
• First Incubation Time	: 15 min. at 37°C.
• Reagent 3 Volume	: 50 microliters (0.05 ml).
• Second Incubation	: 5 min. at 37°C.
• Light Path	: 1.0 cm.
• Zero setting with	: Distilled Water.



CREATININE

END POINT & KINETIC

PROCEDURE :

PIPETTE INTO TEST TUBE	STANDARD (ST)	TEST (TS)
WORKING REAGENT (ml)	1.0	1.0
STANDARD (ml)	0.1	-
SAMPLE (ml)	-	0.1

Mix well and incubate for 15 min at 37°C or (25-30 min. at room temperature).

Record the absorbance of Standard (STD₁) and Test (TS₁) at 510 nm (505 -530 nm or with GREEN FILTER) against Distilled water.

3 CREATININE (ml)	0.05	0.05
-------------------	------	------

Mix well and keep for 5 minutes at 37°C (or 10 min at a room temperature) and record the absorbance of Standard (STD₂) and Test (TS₂) at 510 nm (505 -530 nm or with GREEN FILTER) against Distilled water.

TEST RESULTS

Creatinine concentration (mg/dl) = $\frac{TS}{STD} \times 2$

Where

TS = Corrected absorbance of sample = TS₁-TS₂

STD = Corrected absorbance of Standard = STD₁ -STD₂

INTERFERENCE:

As per studies carried out for interference, following results were obtained.

- No Interference from Hemoglobin upto 50 mg/dl.
- No Interference from free Bilirubin upto 12 mg/dl.
- No Interference from Lipemic (Measured as Triglycerides) upto 500 mg/dl.

NORMAL VALUES: (FOR SERUM)

Serum Creatinine : MEN : 0.9 - 1.4 mg/dL

WOMEN : 0.6 - 1.2 mg/dL

LINEARITY :

This method is linear upto 12 mg/dL. For sample values higher than linearity limit, dilute the samples suitably with 0.9 % saline and repeat the assay. Apply dilution factor to obtain test results.

CREATININE ESTIMATION IN URINE:

For Creatinine estimation in urine, dilute the sample suitably with distilled water and follow the procedure to calculate test result by applying dilution factor. A dilution of 1:50 or 1:100 is suggested.

NORMAL VALUES:

Urine Creatinine: MEN: 1.0 – 2.0 gms/24 hrs

WOMEN : 0.8 – 1.5 gms/24 hrs

REFERENCE:

KAPLAN A., SZABO, L.L., Clinical Chemistry : Interpretation and Techniques, Lea and Febiger, Philadelphia (1983).

