Creatinine-k

Jaffe's Method (Kinetic & End Point Method)

Code : 11015 (2 x 100 ml)

(For the Analyser/ Colorimetric estimation of Creatinine in Serum & Urine)
In VITRO USE only

SUMMARY & EXPLANATION OF TEST :

Serum Creatinine determination is mainly used for the diagnosis of renal diseases. 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. In the kinetic method, a delay, before the picrate creatinine complex formation is monitored, minimises interference from the fast reacting substances such as keto acids and hence subsequent measurements upto 120 seconds, largely refer to true creatinine values only. Other advantages of the kinetic reaction include no deproteinisation, 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.

REAGENTS :

		2 x 100 ml
1.	Picric acid Reagent	100 ml.
2.	Alkaline Buffer Reagent	100 ml.
3.	Standard Creatinine (2mg%)	10 ml.
4.	Acid Reagent	10 ml.

The reagents are stable at room temperature till the expire date mentioned on the label.

WORKING REAGENT PREPARATION :

Mix equal volumes of Picric Acid Reagent(1) & Alkaline Buffer Reagent(2). Working Reagent is stable for 10 days in a Brown Bottle at room temperature.

SAMPLE :

Serum, Plasma or Urine can be used.

1. Urine should be of 24 hours collection. Dilute Urine specimen 1: 100 using distilled water before use.
2. Hemolysed or lipemic serum should not be used, as it may give erroneous results.
3. Creatinine in serum is stable for at least two days at room temperature and one week at 2-8^{0}C or much longer at-20^{0}C.

EXPECTED RANGE :

	Serum Creatinine	Urine Creatinine
Male	0.9 - 1.5 mg%	1.1 - 3.0 gm
Female	0.8 - 1.3 mg%	1.0 - 1.8 gm

LINEARITY :

This method is linear upto 20 mg%. Samples exceeding 20 mg% should be diluted and reassayed. The result has to be multiplied by the dilution factor.

INSTRUCTIONS :

1. Picric acid reagent do not pipette by mouth.
2. Adherence to the reaction time is very critical and should be followed meticulously.
3. For urine creatinine estimation make sure to dilute urine sample 1: 100.
4. For end Point Method strictly follow the time.

DIRECTIONS FOR USE ON ANALYSERS :

Reaction Type	:	Kinetic with std
Wave Length	:	520 nm (green filter)
Incubation Temp	:	Room temperature
Incubation Time	:	30 Secs.
Read Time	:	60 Secs.
No. of readings	:	2
Sample volume	:	100 µl
Reagent volume	:	1 ml
Standard	:	2 mg%
Linearity	:	20 mg%
Unit	:	mg %

PROCEDURE :

KINETIC METHOD

1. Pipette into a clean dry cuvette

Working reagent	1.0 ml
Serum/ Diluted urine/ standard	0.1 ml

2. Mix well and start stopwatch. Read initial absorbance A_0 exactly after 30 seconds for test and for standard.
3. Read another absorbance A_1 for test and for standard exactly 60 seconds later
4. Calculate change in absorbance for test and standard.
5. For test $\Delta AT = A_1T - A_0T$
   For standard $\Delta AS = A_1S - A_0S$

CALCULATIONS :

a) Serum Creatinine in mg% (Std. Conc.) $= \dfrac{\Delta AT}{\Delta AS} \times 2$

b) Urine Creatinine in gm/lit $= \dfrac{\Delta AT}{\Delta AS} \times 2$ (Std. Conc.)

c) Urine Creatinine in gm /24 hours = (b) X 24 hours urine volume in litres.
SI Conversion factor
µ / L = mg% X 88.4

END POINT METHOD

Pipette into clean dry test tubes labelled standard(S) and Test (T)

	S	T
Working reagent	1.0 ml	1.0 ml
Standard	0.1 ml	--
Serum/ Diluted urine	--	0.1 ml

Mixwell and allow it to stand at R.T. for 5 mins. Read absorbance for standard AS_0 and Test AT_0 against distilled water at 520 nm (or) green filter in spectrophotometer.

Acid Reagent	0.05 ml	0.05 ml

Mixwell and allow it to stand at R.T. for 5 mins. Read absorbance for standard AS_1 and Test AT_1 against distilled water at 520 nm (or) green filter in spectrophotometer.

CALCULATION :

$\Delta AS = AS_0 - AS_1$

$\Delta AT = AT_0 - AT_1$

a) Serum Creatinine $= \dfrac{\Delta AT}{\Delta AS} \times 2$ (Std. Conc.)

NOTES : ★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY :

1. Bowers, L.D. (1980) Clin. Chem. 26 : 551.
2. Bowers, L.D. & etal (1980) Clin. Chem. 26 : 655.

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