

## Serum creatinine: conventional and compensated kinetic method with enzymatic method

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### Abstract

**Introduction:** The overall magnitude and pattern of chronic kidney disease (CKD) in India has been studied sporadically. The estimated glomerular filtration rate (eGFR) is valuable in identifying patients at risk of developing nephropathy. However, calculation of the eGFR from serum creatinine levels is not without limitations

**Aim:** The aim of this study was to compare analytical performance and workability of the enzymatic method vs kinetic method (both conventional and compensated) for serum creatinine for routine use and to compare the effects of some common interfering substances like bilirubin on the enzymatic method vs kinetic Jaffe's method.

**Materials and Method:** We measured Serum creatinine by enzymatic, conventional and compensated kinetic creatinine measurement method in 120 samples having bilirubin >1mg/dl.

**Results:** Relationship between Jaffe's kinetic semi-automated method and enzymatic method on Cobas Integra 400 plus for serum creatinine shows regression coefficient 0.88 which means two methods are not correlate with each other. Compensated Jaffe's kinetic method and enzymatic method shows regression coefficient 0.95 which means two methods are correlate with each other and the differences between two methods in Bland-Altman plot shows that compensated Jaffe's kinetic method have negative bias but differences between two methods are less (scatter reading) and constant throughout concentration range.

**Conclusion:** There is good agreement and good comparability of the compensated kinetic Jaffe's method with the enzymatic method than conventional picric acid Jaffe's kinetic method we found that when serum bilirubin was high and serum creatinine was measure with conventional picric acid Jaffe's kinetic method and compensated picric acid Jaffe's kinetic method gives false low creatinine.

**Keywords:** Enzymatic, Conventional and Compensated Kinetic.

### Introduction

Creatinine is a waste product of creatine. Serum creatinine is widely used as an index of renal function and also serum creatinine widely used to estimate the glomerular filtration rate (GFR).<sup>(1)</sup> The estimated glomerular filtration rate (eGFR) is used to detect early kidney damage and diagnose chronic kidney disease (CKD), and to monitor kidney status such as the case of patients undergoing procedures requiring administration of radiographic contrast media. eGFR is a calculation based on the results of a blood creatinine test.<sup>(2)</sup> CKD has been documented as a major global public health problem.<sup>(3,4,5)</sup>

Creatinine, along with eGFR, is usually used to screen people with known CKD and those with high risk for developing CKD for example diabetes mellitus and hypertension. The calculation of the eGFR from serum creatinine levels have some limitations. There are major analytical bias and positive and negative interference by chromogens in many commercially available serum creatinine assays is a source of major concern.<sup>(6,7)</sup> Also serum bilirubin interfere (negative bias) with creatinine estimation by picric acid method. And the occurrence of renal failure along with high serum bilirubin is common.

Bilirubin inhibit the reaction between creatinine and alkaline picrate. Bilirubin causing a decrease in absorbance because under alkaline conditions bilirubin

is oxidized to biliverdin, that's why bilirubin interference creatinine estimation.<sup>(8)</sup>

The current situation in Indian clinical laboratories is that the picric acid creatinine measurement method (both conventional and compensated) coexists with the enzymatic creatinine measurement method. In this work attention was focused on the impact of the inherent differences between these three methods on the applicability of the equations. In addition, different measurement systems with various instruments, reagents and calibrators, have distinct ways of monitoring the magnitude measured by creatinine resulting in different results. Whether all these differences affect the applicability of the equations in different laboratories must be determined. Creatinine measurement in this study consisted of three measurement systems.

Enzymatic serum creatinine measurement is costly. But when compared with the picric acid method, this process can overcome the interference of false creatinine materials such as vitamin C, pyruvate, acetone, and glucose. Thus, enzymatic serum creatinine measurement produces more accurate measurement results.<sup>(9)</sup> Therefore we have chosen enzymatic IDMS Traceable serum creatinine method as gold standard.

The aim of this study was to compare analytical performance and workability of the enzymatic method and kinetic method (both conventional and

compensated) for serum creatinine for routine use and to compare the effects of some common interfering substances like bilirubin on the enzymatic method and kinetic Jaffe's method.

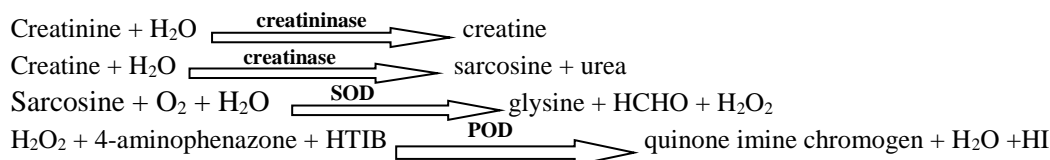
### Materials and Method

The study was carried out at Clinical Biochemistry department of central diagnostic laboratory in Shree Krishna hospital, Karamsad. The procedures followed were in accordance with the Clinical Research Ethics Committee of Pramukh Swami Medical College, Karamsad. The study subjects will include the entire patients, who come to test for kidney function of age group between >18 and <60 years attending at Shree Krishna hospital, Karamsad.

As a routine procedure, the 3ml venous blood were collected in plain vial. The samples were centrifuged at 3000 rpm for 15 min to obtain serum and were analysed for creatinine on the same day.

Estimation of serum creatinine was carried out within 4-6 hours of collection on fully automated COBAS Integra 400+ clinical chemistry analyzer by compensated jaffe method and by enzymatic method. Serum creatinine estimated on RA-50 semi-automated analyzer by jaffe kinetic method. Data were noted in study proforma.

### Methodology



The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration. It is determined by measuring the increase in absorbance at 552 nm. This method has been standardized against IDMS (isotope dilution mass spectrometer).

The 2 levels of quality control materials used in this study were supplied from Randox. We also estimated Serum Total Bilirubin by azobilirubin method.

We determined the median difference between the two methods and analysed the agreement between them. The relationship between the two methods was also compared by regression analysis.

The Statistical Package for the Social Sciences (version 11.0; SPSS) was used for statistical analyses. Linear regression model was used to establish correlation coefficients.

### Results

**Table 1: General Statistics**

Gender	Total Number of Sample	Median				
		Age	Total Bilirubin	JAFFE kinetic (Picric acid) Semi-auto analyzer	Compensated JAFFE kinetic Cobas Integra 400 +	Enzymatic Method Cobas Integra 400 +
Female	40	50	5.4	1.6	1.68	1.79
Male	80	48	7.9	1.23	1.50	1.66
Total	120	48.5	5.7	1.3	1.58	1.69

#### On RA-50 semi autoanalyzer:

1) **Method:** Jaffe rate kinetic method

- **Principle:** creatinine in alkaline solution react with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

#### On cobas integra 400:

1) **Method:** Jaffe compensated method

- **Principle:** buffered kinetic jaffe reaction without deproteinization. Compensated for serum/plasma.

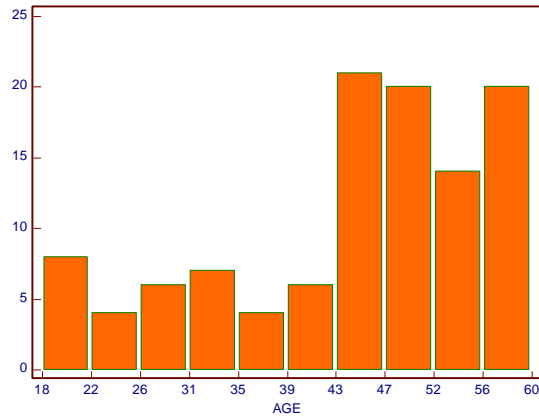
In alkaline solution creatinine reacts with picrate to form a yellow-red complex.

Creatinine + Picric acid → Yellow-red complex

The rate of the dye formation (color intensity) is directly proportional to the creatinine concentration in the specimen. It is determined by measuring the increase in absorbance at 512 nm. Serum and plasma samples contain proteins which react non-specifically in the jaffe method. For compensation of serum and plasma results, values are automatically corrected by 18 micromol/l (-0.2 mg/dl).

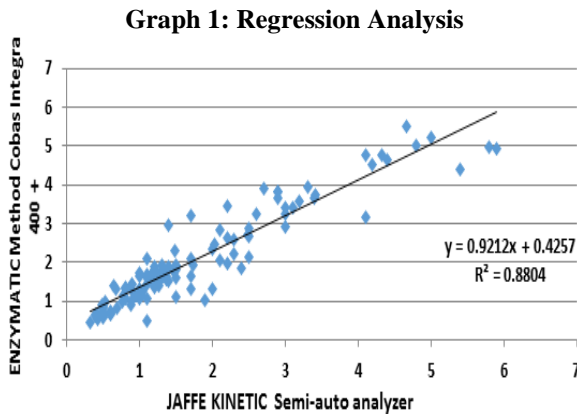
2) **Method:** Enzymatic method

- **Principle:** The enzymatic method is based on the established determination of hydrogen peroxide after conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase. The liberated hydrogen peroxide reacts with 4-aminophenazone and HTIB to form a quinone imine chromogen.

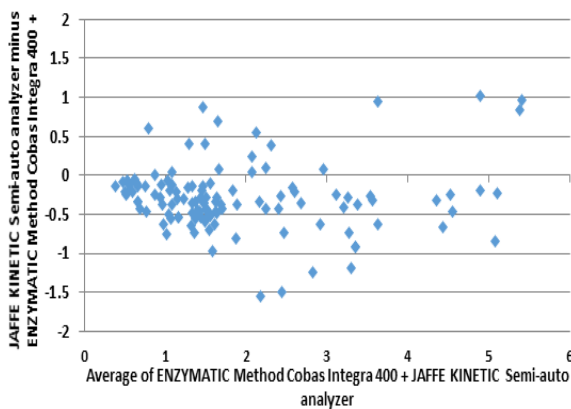


**Fig. 1: Age wise frequency distribution**

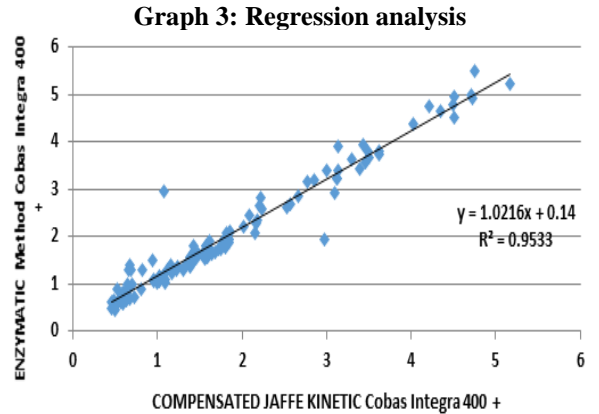
Graph 1, 2: Relationship between Conventional Jaff’s kinetic method and enzymatic method on Cobas Integra 400 plus for serum creatinine.



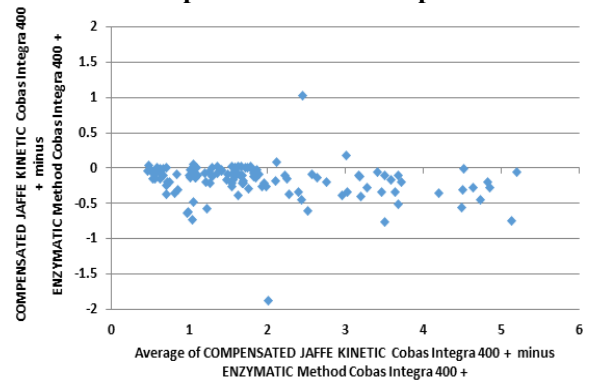
**Graph 2: Bland-Altman plot**



Graph 3, 4: Relationship between Compensated Jaff’s kinetic method and enzymatic method on Cobas Integra 400 plus for serum creatinine



**Graph 4: Bland-Altman plot**



**Discussion**

In the present study we find that bilirubin interfere in the estimation of creatinine by conventional and compensated Jaffe Kinetic method. Most of the small and median size laboratory use semi-autoanalyzer that’s why comparison require with fully autoanalyzer. We have a NABL accredited laboratory so we have a scope to compare semi-autoanalyzer with fully autoanalyzer that’s why take this comparison.

Samples for comparison selected randomly and test for normality were rejected therefore instead of taking mean we have calculated median. Data shows median age for female, male and total were 50, 48 & 48.5 years respectively. Median total bilirubin was 5.7 mg/dL and median serum creatinine for JAFFE KINETIC (Picric acid) Semi-autoanalyzer, COMPENSATED JAFFE KINETIC Cobas Integra 400 plus and ENZYMATIc Method Cobas Integra 400 plus were 1.3, 1.58 and 1.69 mg/dL respectively, which is nearer to upper reference limit for serum creatinine value.

Relationship between Jaff’s kinetic semi-automated method and enzymatic method on Cobas Integra 400 plus for serum creatinine Graph 1 shows regression analysis between Jaff’s kinetic semi-automated method and enzymatic method on Cobas Integra 400 plus with slope is 0.92; intercept 0.42 and regression coefficient 0.88 which means two methods are not correlate with each other and the differences is more evident in Graph 2 Bland-Altman plot that Jaff’s kinetic semi-automated

method have negative bias and differences between two methods increases (scatter reading) with increasing serum creatinine concentration.

Graph 3 shows regression analysis between Compensated Jaff's kinetic method and enzymatic method both on Cobas Integra 400 plus with slope is 1.02; intercept 0.14 and regression

coefficient 0.95 which means two methods are correlate with each other and the differences between two methods in Graph 4 Bland-Altman plot shows that compensated Jaff's kinetic method have negative bias but differences between two methods are less (scatter reading) and constant throughout concentration range. It also shows two reading very far from midline, which are might be outlier and if eliminate can further improve method comparison data. When bilirubin was present in the serum samples (bilirubin > 1 mg/dl), the median difference between enzymatic and compensated kinetic Jaffe's method was statistically not significant (-0.11).

Hence in routine compensated kinetic Jaffe's method can be used. Creatinine estimation is a index of renal function. As show the result estimation of creatinine by Compensated JAFFE Kinetic median Percentage difference is 6.7% and by Conventional JAFFE Kinetic it is 17.76% from the Enzymatic Method. So both method give false low value of creatinine hence care should be taken before reporting of creatinine with bilirubin >1mg/dl.

In this study we employed a small sample size and could test effects of only one interfering substances. Future studies will aim at analyzing the effects of many more interfering substances.

## Conclusion

In this study we found that when serum bilirubin was high and serum creatinine was measure with conventional picric acid Jaff's kinetic method and compensated picric acid Jaff's kinetic method gives false low creatinine that indicate we missed cases having kidney failure when compared with serum creatinine measure with enzymatic IDMS Traceable. There is good agreement and good comparability, of the compensated kinetic Jaffe's method with the enzymatic method than conventional picric acid Jaff's kinetic method.

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