Clinical Evaluation of Analytical Variations on Creatinine Measurement During Delayed Serum Separation by Jaffe and Enzymatic Methods with Different Temperature and Time Interval

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ABSTRACT

Introduction: The most common methods used in the laboratories for measuring serum creatinine level are Jaffe and enzymatic methods. Compared to Jaffe method, enzymatic method is more expensive but less susceptible to interferences. Interferences lead to misleading of test results and lead to misdiagnosis. The overall risk associated with the Jaffe method depends on the probability of misclassification of Chronic Kidney Disease. In practice, very few routine specimens can be processed within the time interval manually. Considerable delay occur in sample delivery, either because of human errors or improper handling procedures. In that cases, serum contact with clot may exceed 24 hours, result in clinically significant changes of analytes present in the serum. Delays in serum separation can cause significant increases in measured creatinine by the commonly used Jaffe method. This study will help to give an insight on the effects of delayed serum separation and to find out an appropriate method, when delays occur and help to determine the maximum possible storage time to store the blood sample as whole blood before the analysis at refrigerator and at room temperature.

Objective: Objective of this study is to analyzing the effect of delayed serum separation on creatinine measurement by Jaffe method with an enzymatic method.

Methodology: The study was carried out from August 2018 to July 2019. Data collection was carried out in June 2019. The blood samples were collected from the voluntary healthy male and female students from the Faculty of Medicine, University of Jaffna. Two set of tubes were kept at room temperature (≃27-29℃) and refrigerator (2-8℃) respectively. After 2 & 6 hours and 1, 2 and 3 days of storage, clotted samples were centrifuged at 3000 rpm for 10 minutes and serum was separated. Pooled serum was prepared by mixing required amount of serum samples on each time interval for both tubes at room temperature (≃27-29℃) and (2-8℃) refrigerator. Then the creatinine concentration was measured by using Jaffe method and Sarcosine Oxidase enzymatic method. Pooled serum was prepared by mixing all serum samples at each time interval. The data and results obtained were analyzed using descriptive statistics such as tables, graphs, mean comparison, standard deviation. Data was entered in Statistical Package for Social Sciences (SPSS) version 23. The *p*-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results: Serum samples collected as soon as the collection of the blood were analysed for the baseline creatinine concentration and considered as 0 hour. The mean creatinine was 1.213 (±0.004) mg/dL by Jaffe method and 1.065 (±0.007) mg/dL by Sarcosine Oxidase enzymatic method (Table **1**). The reference range of serum total creatinine in adult individual is is 0.9-1.3 mg/dL (80-115 µmol/L) (Burtis *et al*., 2008). Total creatinine concentration of the serum samples prepared from the blood samples stored at room temperature were measured at specified time intervals. The mean serum creatinine concentrations (mg/dL) measured by

Jaffe method and Sarcosine Oxidase enzymatic method explained in the Table **1**. Total creatinine concentration of the serum samples prepared from the blood samples stored in the refrigerator were measured at specified time intervals. The mean serum creatinine concentrations (mg/dL) measured by Jaffe method and enzymatic method explained in the Table **2**.

Conclusion: There were no statistical difference ($p > 0.05$) of creatinine concentration that occurred up to 1 day of sample storage by Jaffe method and up to 2 days by enzymatic method. Whole blood sample stored at room temperature (27-29˚C) for measurement of serum creatinine by using Jaffe method is acceptable when samples stored maximum of up to 6 hours only. According to this study, whenever delays occur keeping the blood samples in the refrigerator (2-8˚C) is better than storing at room temperature (27-29˚C). Also measuring serum creatinine by the enzymatic method will minimize the errors than using the Jaffe method and it will lead to the release of accurate test results.

Table 1. Creatinine concentration of serum prepared from the blood samples stored at room temperature by Jaffe's method and Sarcosine Oxidase enzymatic methods.

Time	Jaffe's method Creatinine concentration (±SD) (mg/dL)	P# value	Change in Creatinine (%)	Enzymatic method Creatinine concentration (±SD) (mg/dL)	P# value	Change in Creatinine (%)
0	1.213 (±0.004)	-	-	1.065 (±0.004)	-	-
2	1.227 (±0.004)	0.990	1.14%	1.108 (±0.006)	0.040	3.89%
6	1.472 (±0.005)	0.010	17.53%	1.217 (±0.004)	0.150	12.48%
1 day	1.735 (±0.014)	0.000	30.08%	1.310 (±0.036)	0.000	18.70%
2 days	2.120 (±0.064)	0.000	42.78%	1.410 (±0.028)	0.000	24.46%
3 days	2.240 (±0.069)	0.000	45.84%	1.473 (±0.005)	0.000	27.69%

Table 2. Creatinine concentration of serum prepared from the blood samples stored in the refrigerator by Jaffe's method and Sarcosine Oxidase enzymatic methods.

Time	Jaffe's method Creatinine concentration (±SD) (mg/dL)	P# value	Change in Creatinine (%)	Enzymatic method Creatinine concentration (±SD) (mg/dL)	P# value	Change in Creatinine (%)
0	1.213 (±0.004)	-	-	1.065 (±0.004)	-	-
2	1.246 (±0.014)	0.960	2.65%	1.092 (±0.009)	0.960	2.47%
6	1.324 (±0.006)	0.270	8.38%	1.100 (±0.007)	0.900	3.18%
1 day	1.487 (±0.007)	0.010	18.43%	1.187 (±0.010)	0.070	10.27%
2 days	1.670 (±0.085)	0.000	27.36%	1.230 (±0.042)	0.020	13.41%
3 days	1.720 (±0.068)	0.000	29.48%	1.324 (±0.007)	0.000	19.56%

Keywords: Creatinine, Chronic Kidney Disease, Enzymatic assay, Jaffe method, Sarcosine Oxidase, Interferences

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