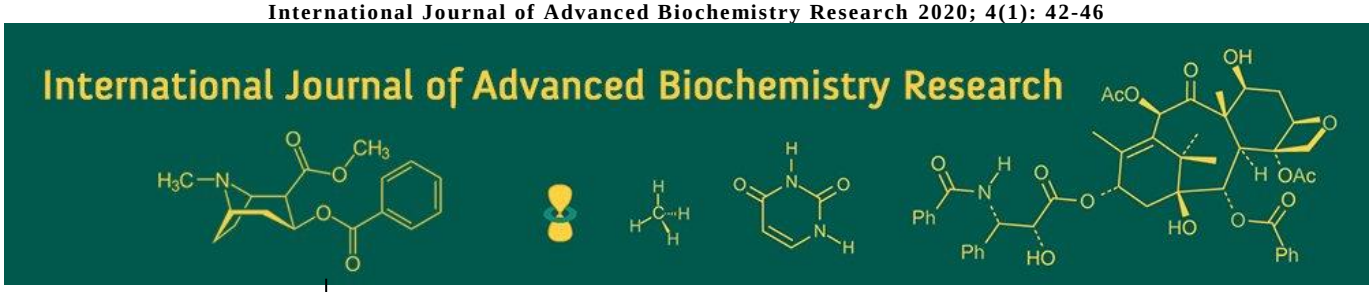

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Comparison of 150mmol/L and 250mmol/L concentration of sodium hydroxide in Jaffe's two point method for serum creatinine measurement with respect to linearity of assay

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Abstract
Objectives: Objective of the study is to perform linearity check for Creatinine measurement with Jaffe's two point method using CLSI EP-6 guideline for 150 mmol/L, 250mmol/L NaOH concentration in final reagent mixture.
Material & Method: Leftover serum of samples received by the laboratory, devoid of patient identification, was used to make pool of serum. Serum creatinine reagents have NaOH of 150 mmol/L, 250 mmol/L were prepared. Serial dilution of pooled serum spiked with creatinine powder was prepared. Creatinines were measured in series of dilution. Linear regression analysis of each dilution series to find linearity limit was performed.
Result & Discussion: This study evaluated Linearity difference plot using CLIA criteria (15% or 0.3 mg/dl whichever is higher) for creatinine at 95% confidence limit.7.5% of result or 0.15 mg/dl were used for plotting Linearity Difference plot. If any the two conditions are satisfied at a given point in the Linearity Difference Plot, the point is considered within linearity. 250 mmol/L NaOH in final reagent mixture gives limit of linearity 23.77mg/dl and 150 mmol/L NaOH in final reagent mixture gives limit of linearity 31.16 mg/dl.
Conclusion: 150 mmol/L final reagent concentration of NaOH gives higher linearity of 31.16 mg/dl, low slop (a=29.23) indicating high sensitivity of the reagent, low y-intercept (b=-0.4578) indicating minimum effect of non-creatinine chromogens; i.e. high specificity than 250mmol/L final reagent concentration of NaOH.

Keywords: Limit of linearity (LoL), NaOH concentration.

1. Introduction
Creatinine measurement in serum is one of the most frequently used laboratory examination in clinical chemistry laboratory. It is used in diagnosis, treatment monitoring and judging prognosis of renal disorders. In order to be useful to the clinicians, the creatinine reporting should be accurate, precise, free from interference. It should have well defined limit of blank, limit of detection, limit of qualification and limit of linearity. It is required to compare NaOH concentration of these reactants to improve its clinical usefulness. This study aims at comparing 150mmol/L and 250mmol/L NaOH concentration in Jaffe's reaction mixture with respect to limit of linearity (LOL). Measurement of Limit of Linearity is essential to device alternate strategy for measuring samples when results are beyond the limit.

Measurement of Limit of Linearity is essential to device alternate strategy for measuring samples when results are beyond the limit.

2. Materials and Methods
Leftover serum of samples received by the laboratory, devoid of patient identification, was used to make pool of serum which had creatinine concentration less than 1.0mg/dl with no visible lipemia, hemolysis and icterus. Higher concentration pool was made by dissolving 25mg creatinine powder in 25ml leftover serum pool. Remaining serum pool was used as low serum pool. Serum creatinine reagent 1 were made by adding SDS 3.47mmol/L and Brij 3gm/L and NaOH 300mmol/L in which final reagent mixture have NaOH reagent mixture have NaOH of 150 mmol/L.

Serum creatinine reagent 1 were made by adding SDS 3.47mmol/L and Brij 3gm/L and NaOH 500mmol/L in which final reagent mixture have NaOH reagent mixture have NaOH of 250 mmol/L.

Creatinine reagent 2 were made by adding picric acid 20mmol/L and Brij 3gm/L. 11 point serial dilution of prepared higher pool with lower pool was done as recommended by CLSI EP-6A guideline [1]. Calibration of each reagent was done by Randox Normal Calibrator (828UN), Randox Abnormal Calibrator (591UE) and Bovine Albumin Blank (5 gm%) whose assigned value was 1.37mg/dl, 4.01mg/dl and 0.0 mg/dl respectively.

Creatinine measurement with each reagent in each member of series of dilution in 4 replicates were performed in Erba XL 640 autoanalyser. Graph of relative concentration vs. observed concentration was plotted in. First, Second and third order polynominals were found for each plot using spreadsheet software. Deviations from linearity (DLi) were evaluated as follows for each point of the plot. DL2i were used to analyse linearity as it was closed to 1. CLIA acceptability criteria was used to analyse the sample.

Results

Following tables and graphs shows results of the study.

Results of Linearity study for 150 mmol/L NaOH reagent

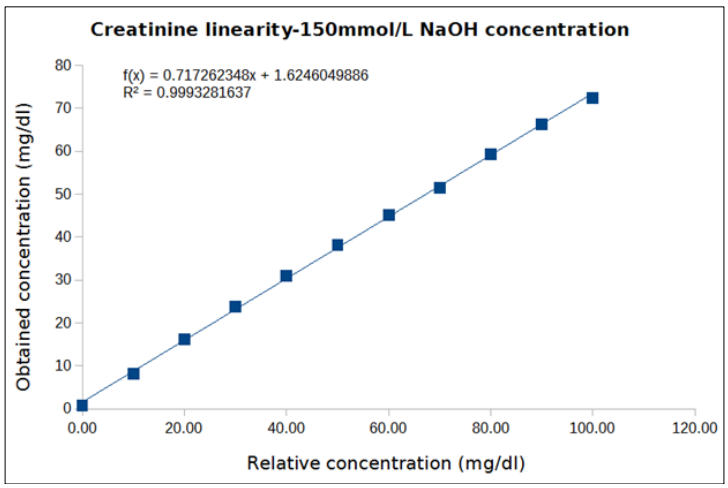

Fig 1: (Illustration 1): Creatinine Linearity-150mmol/L of NaOH

Above graph shows relationship between relative concentration and obtained concentration. Relative concentration is taken from 0 to 100; as per CLSI EP-6A, any values can be used as relative concentrations so far as their relative values remain same [1].

Creatinine Linearity Difference Plot for 150 mmol/L NaOH concentration, Stage-1: As described in CLSI EP-6A, calculations were done for making Linearity Difference plot. The plot requires definition of acceptability criteria. This study evaluated Linearity difference plot using CLIA criteria for creatinine at 95% confidence limit. As CLIA acceptability for creatinine is 15% or 0.3 mg/dl whichever is higher, 7.5% of result or 0.15 mg/dl were used for plotting Linearity Difference plot. If any the two conditions are satisfied at a given point in the Linearity Difference Plot, the point is considered within linearity. Stage-1 is Linearity Difference Plot which includes all the 11 points. Stage-2 is Linerarity Difference Plot which includes only those points which make linearity evaluation acceptable at all points. The calculations of Linearity Difference plot are explained in table 11 for 150 mmol/L NaOH.

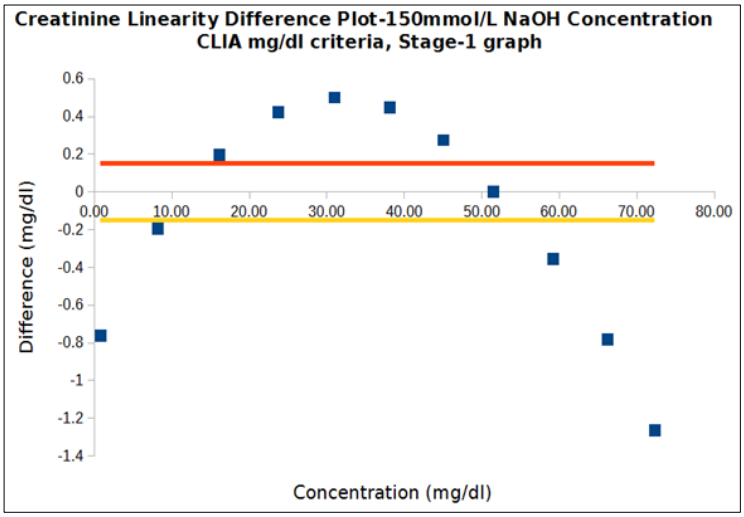

Fig 2: (Illustration 2) Creatinine Linearity Difference plot-150mmol/L NaOH concentration CLIA mg/dl criteria, Stage-1 Above graph shows except point 8, all other points are outside the ±0.15 mg/dl.

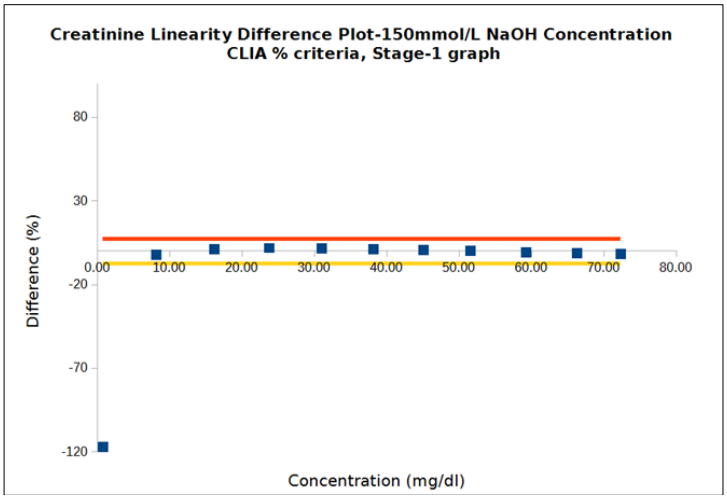

Fig 3: Illustration 3: Creatinine Linearity Difference plot-150mmol/L NaOH concentration CLIA% criteria, Stage-1

Above graph shows that except point 1, all other points are within the ±7.5% limit. By both, % and mg/dl criteria, point 1 is unacceptable, so, the analysis is repeated by removing highest point, one by one till the all the points come within the acceptable criteria.

Creatinine Linearity Difference Plot for 150 mmol/L NaOH concentration, Stage-2

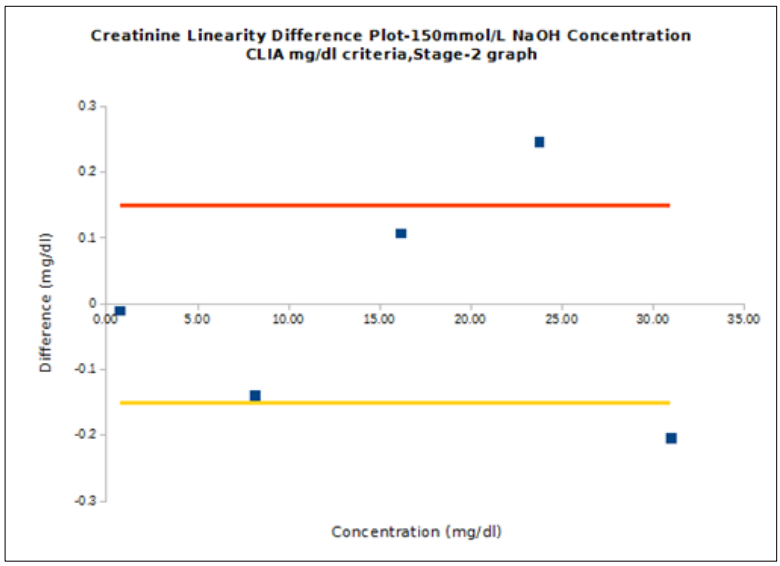

Fig 4: (Illustration 4) Creatinine Linearity Difference plot-150mmol/L NaOH concentration CLIA mg/dl criteria, Stage-2

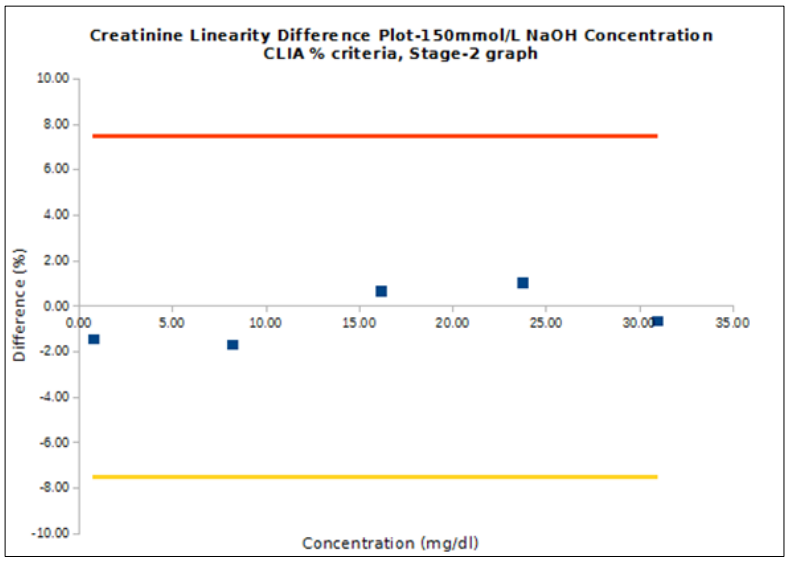

Fig 5: (Illustration 5) Creatinine Linearity Difference plot-150mmol/L NaOH concentration CLIA% criteria, Stage-2

As Shown in above both graphs if first 5 points are used for Linearity Difference Plot, all the point satisfy at least of the two CLIA criteria. Hence, for 150 mmol/L NaOH, method is linear up to 31.16 mg/dl. Similar analysis is performed for 250mmol/L NaOH concentration.

Results of Linearity study for 250 mmol/L NaOH reagent

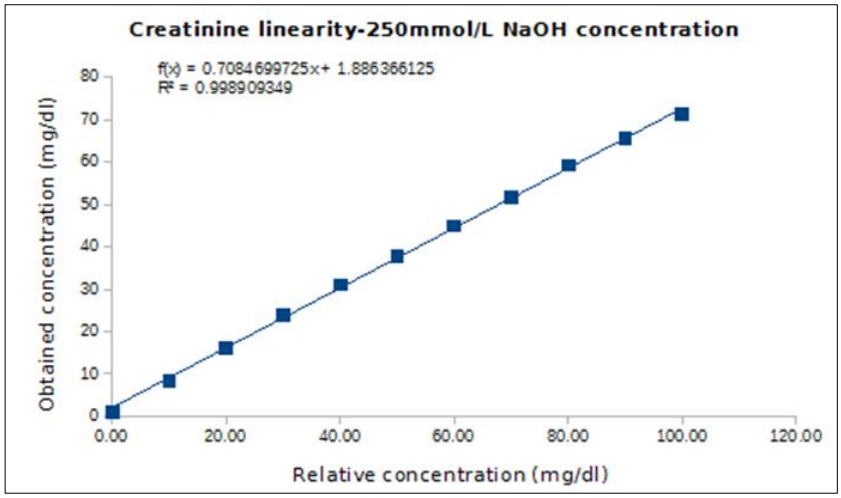

Fig 6: (Illustration 6) Creatinine linearity-250mmol/L NaOH concentration

Creatinine Linearity Difference Plot for 250 mmol/L NaOH concentration, Stage-1

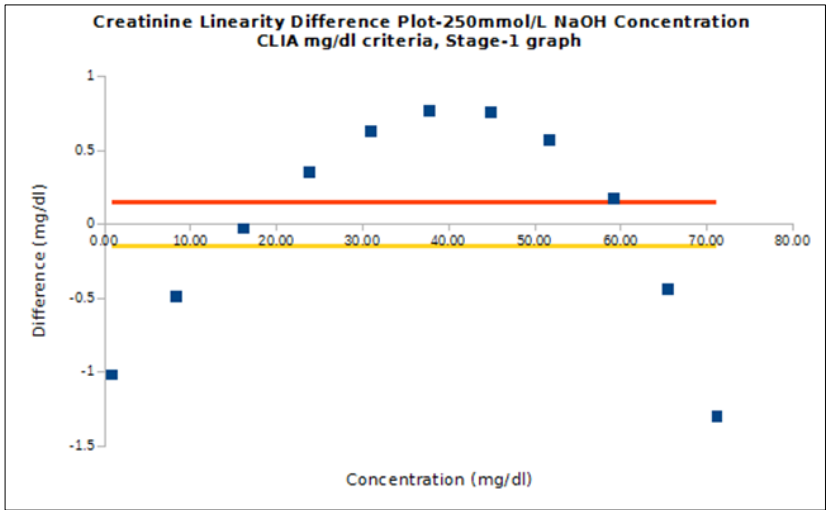

Fig 7: Creatinine Linearity Difference plot-250mmol/L NaOH concentration CLIA mg/dl criteria, Stage-1

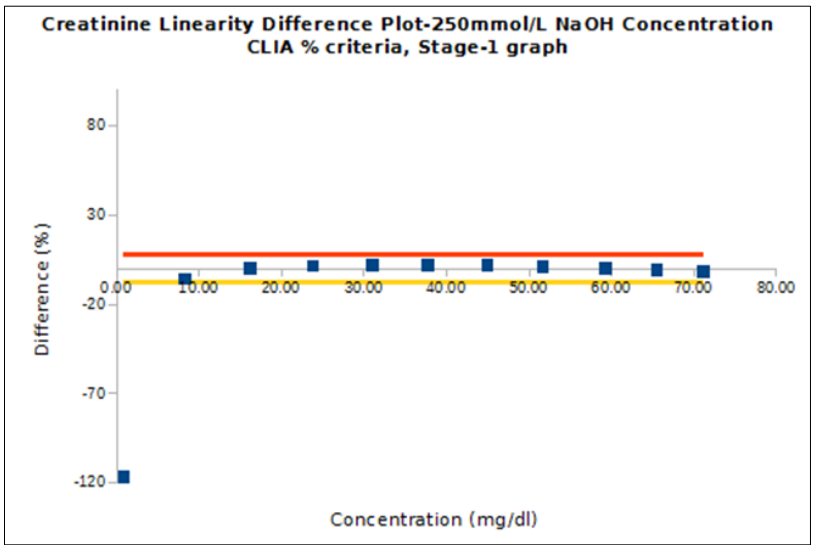

Fig 8: Creatinine Linearity Difference plot-250mmol/L NaOH concentration CLIA mg/dl criteria, Stage-1

Above graph shows that except point 1, all other points are within the ±7.5% limit.Only 1 point is within the 0.15mg/dl. By both, % and mg/dl criteria, point 1 is unacceptable, so, the analysis is repeated by removing higest point, one by one till the all the points come within the acceptable criteria.

Creatinine Linearity Difference Plot for 250 mmol/L NaOH concentration, Stage-2

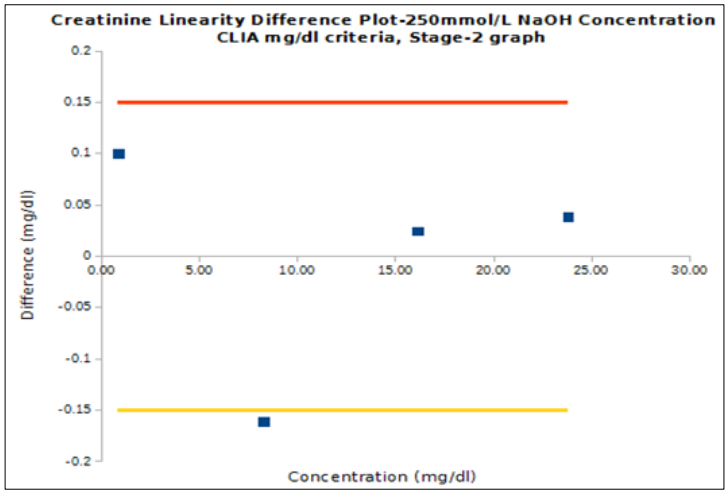

Fig 9: Creatinine Linearity Difference plot-250mmol/L NaOH concentration CLIA mg/dl criteria, Stage-2

As Shown in above both graphs if first 5 points are used for Linearity Difference Plot, all the point satisfy at least of the two CLIA criteria. Hence, for 250 mmol/L NaOH, method is linear up to 23.77mg/dl

Discussion and Conclusion

Table 1: Limit of Linearity for 150mmol/L and 250mmol/L NaOH concentration in final reagent mixture

NaOH Concentration(mmol/L)	Limit of Linearity (LOL) (mg/dl)	Slop (a)	Y-Intercept
150	31.16	29.23	-0.4578
250	23.77	28.27	-0.6996

Above Table shows 150mmol/L reagent has higher linearity than 250mmol/L. Lower Slope of 150mmol/L reagent shows higher sensitivity and lower y-intercept shows lower non creatinine chromogens interference.

Measurement of Limit of Linearity is essential to device alternate strategy for measuring samples when results are beyond the limit.

Two strategies used commonly are 1) Dilution of sample 2) decreasing sample volume. Both strategies involve repeat testing which involves cost. Too low of LoL increases repeat testing, increasing cost. Higher LoL decreases the dilution of sample and sample load.

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