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Comparison of the Effect of Two Different Buffer Systems Used for the Estimation of Serum Alkaline Phosphatase on Interpretation of the Test Results

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ABSTRACT

OBJECTIVES: The commonly used IFCC method for Alkaline Phosphatase estimation is based p-Nitrophenyl Phosphate (pNPP) substrate and either of two buffers 2-amino-2-methyl-1-propanol (AMP) or Diethanolamine (DEA), this study was carried out with the objective of comparing both buffers and to find out any significant impact on interpretation of the results.

METHODOLOGY: This method comparison study was carried out on 75 adult patients' serum samples including normal & abnormal liver function, samples were tested simultaneously with both kits in same analyser. Statistical analysis was done using comparison plot & calculating coefficient of comparision (r) & determination (r2), comparison was done by paired t test & Bland Altmann plot.

RESULTS: The data is distributed normally as shown by Shapiro Wilk p-value 0.52 (α=0.05). Mean & SD with AMP & DEA buffers were 125.95 +/-81.35 U/L & 260.12 +/-206.39U/L respectively. Pearson's correlation coefficient (r) 0.9936 & coefficient of determination (r2) 0.9872 shows very strong direct relationship in the results. In paired t test, t is 9.22 & p is<0.0001shows significant difference in the results with both the buffers. Bland–Altman plot shows proportional bias ie the difference in the results increases with higher values. We derived a conversion factor of 2.06 to convert the values obtained from one buffer to another.

CONCLUSION: This study concludes that both the buffers cannot be used interchangeably and switching over will require either a different reference range or use of a conversion factor which every lab should derive for themselves to avoid false interpretation of patients results.

Introduction

Alkaline Phosphatase (ALP) is a crucial enzyme analyzed in the clinical laboratory via spectrophotometry. The two most commonly available reagent kits for ALP estimation both utilize p-Nitrophenyl Phosphate (pNPP) as the substrate. However, they differ in the buffer system employed: 2-amino-2-methyl-1-propanol (AMP) or Diethanolamine (DEA).

Despite both kit types yielding similar results in internal and external quality control checks, a discrepancy has been noted when testing patient serum samples. This variance can lead to misinterpretation of test results by clinicians, particularly when comparing results from different laboratories or even within a single laboratory that employs both buffer systems. To address this issue, test results where two different reagent kits have two different reference ranges require careful interpretation.

Aim and Objectives

Aim:

To evaluate the possible difference in the test results between the two buffer methods (AMP and DEA) and determine whether the change of buffer will affect the patient results and the medical decisions based on those results.

Objectives:

- To estimate the ALP levels in patient samples using both types of reagent kits.
- To compare and correlate the test results.
- To assess the suitability of both kits for interchangeable use.

Methodology

This method comparison study included 75 adult patients' serum samples, encompassing both normal and abnormal liver function profiles.

- **Sample Testing:** All samples were tested simultaneously with both the AMP and DEA kits on the same analyzer on the day of sampling.
- **Measurement Range:** The selected samples covered the entire clinically meaningful measurement range of ALP.
- **Batch Analysis:** To mimic real-world conditions, samples were measured over several days and in multiple batches.

Statistical Analysis:

The following statistical tests were performed on the data:

- **Shapiro-Wilk test:** To assess the normality of the data distribution.
- **Paired t-test:** To determine the statistical significance of the differences between the test results from the two kits.
- **Correlation analysis:** The degree of association was assessed using the correlation coefficient (r) and the coefficient of determination.
- **Bland-Altman plot:** To visualize and describe the trend of bias between the two buffers.

Results

Normality of Data: The Shapiro-Wilk p-value was 0.5244 (alpha=0.05), indicating that the data is normally distributed.

Mean Values:

- Mean with AMP kit: 125.95 +/- 81.3 IU/L.
- Mean with DEA kit: 260.12 +/- 206.39 IU/L.

Conversion Factor: The results from the DEA kit are approximately 2.06 times higher than those from the AMP kit.

Paired t-test: The calculated 't' value was 9.22, with a p-value of <0.0001 (significant at $p < 0.01$). This demonstrates a significant difference between the results obtained with the two buffer systems.

Correlation Analysis:

- The Correlation coefficient (r) was 0.99, indicating a very strong direct relationship between the two buffers.

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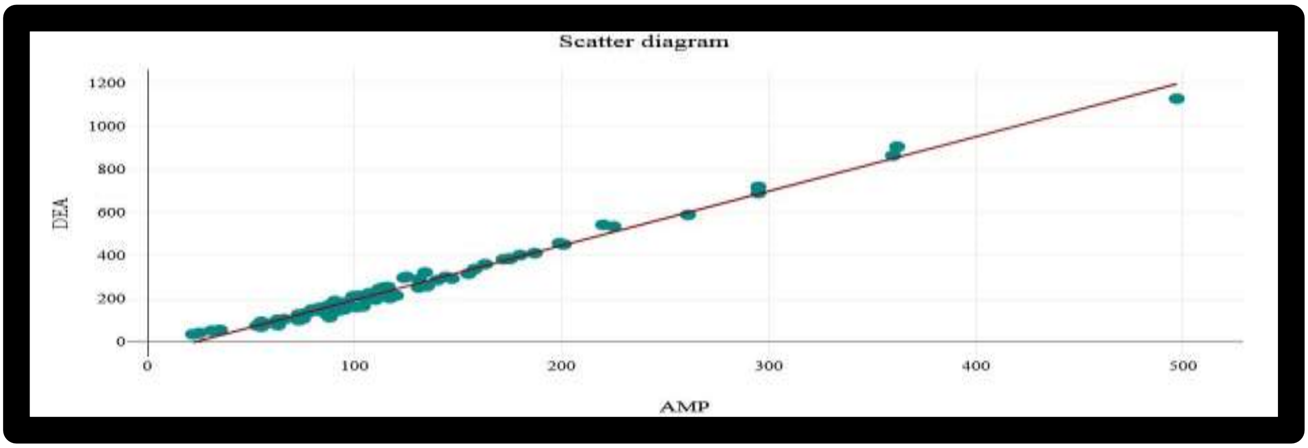
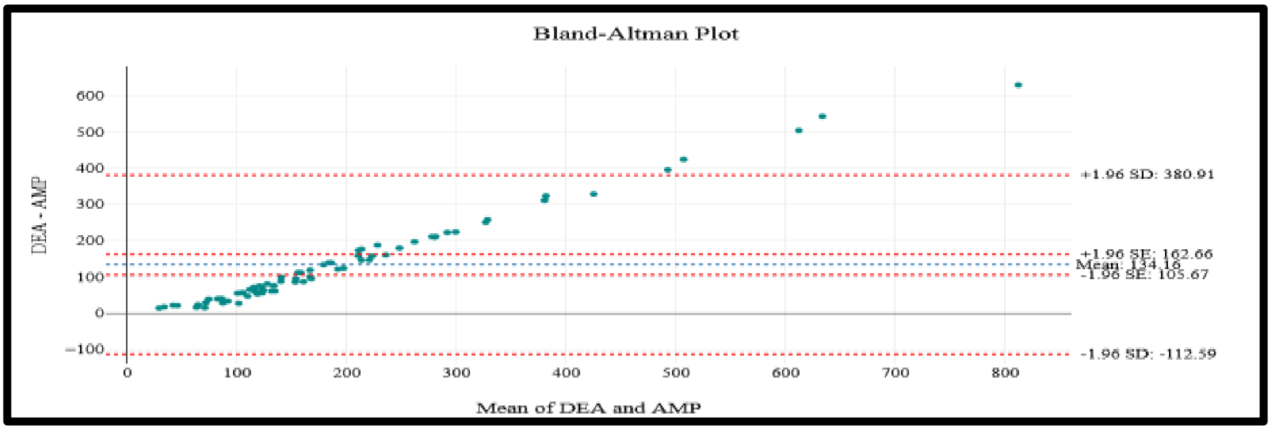
- The Coefficient of determination was 0.987, meaning that 98.7% of the variance is shared between the two variables.

Bland-Altman Plot: The plot revealed a proportional bias, where the difference between the buffer results is narrow at lower mean values and increases as the value of the mean increases.





Discussion

The activity of an enzyme, such as ALP, is known to be affected by the type of buffer used in the analysis. Buffers are generally classified as inert (e.g., carbonate, barbital), inhibiting (e.g., glycine, propylamine), or activating. Both AMP and DEA are classified as activating buffers. In the presence of activating amino alcohols like AMP, DEA, or TRIS, the rate of phosphatase enzyme action is enhanced because they act as phosphate-accepting buffers.

The IFCC recommends the AMP buffer system as the most optimized method for ALP estimation, noting its optimal concentrations of Mg and Zn, appropriate pH range for ALP activity, and the inclusion of N-hydroxyethylethylenediminetriacetic acid (HEDTA) as a chelating agent. However, both IFCC and WHO allow individual laboratories to choose between AMP and DEA buffer systems, provided the corresponding reference ranges are used.

The conversion factor derived In this study is 2.06, suggesting that the DEA method yields results roughly double those of the AMP method. This outcome is similar to a study by Rajeswari S et al., who established a factor of 2.47 to convert AMP results to DEA values. Conversely, another study noted that four human isoenzymes showed varying degrees of inactivation during preincubation with AMP buffers, while no loss of activity was observed with six DEA buffer samples.

Conclusion

The results obtained with the DEA buffer system are approximately twice the results obtained with the AMP buffer system. This study concludes that the two buffer systems cannot be used interchangeably. Switching between them requires either the adoption of a different reference range or the use of a conversion factor, which each laboratory should derive internally. This is essential to avoid the false interpretation of patient results.

Strengths and Limitations

Strengths:

This study highlights the limitations of the current quality control system, which produces similar results for both buffer systems in an artificial matrix but shows a significant difference when testing real patient samples.

Limitations:

- The reagent kits used in the study were sourced from different manufacturers.
- The study utilized a limited sample size.