Original Article

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Biochemistry Section

Comparison of Clinical Chemistry Analysers ERBA XL-640 vs ERBA XL-1000 for Glucose Estimation

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ABSTRACT

Introduction: With advance in technology, many instruments with wide variety of principle or technologies are available in market and in laboratory to conduct sample processing in time bound period. To fulfill the laboratory quality goal, it is essential that result matching be obtained in all used biochemical analysers for examinations. Documented evidence of method/instrument comparison can be used in future for quality improvement purposes.

Aim: To assess and compare equivalence and harmonisation in glucose results produce by ERBA XL-640 vs ERBA XL-1000 in clinical biochemistry laboratory.

Materials and Methods: A comparative study was carried out by results comparison of glucose measurements obtained in two automated systems ERBA XL-640 and ERBA XL-1000 in Clinical Biochemistry laboratory at Surat Municipal Institute of Medical Education and Research between June 2021 to October 2021, based on protocol EP09-3A of result harmonisation and review article-method comparison. The results value were compared according to the total allowable error in Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). Results were analysed by visual and quantitative analysis method Regression analysis and Bland-Altman plot, Method evaluation chart (Normalised MEDx chart).

Results: Total of 48 samples were run within the batches and between the batches and quantitative analysis of difference was done. It is acceptable at medical decision level 40 mg/dL, 120 mg/dL and 180 mg/dL with comparison to total allowable error.

Conclusion: The present study concluded that the comparison of ERBA XL-640 and ERBA XL-1000 for glucose examination by end point method {Glucose Oxidase-Peroxidase (GOD-POD) method} is acceptable and can be used interchangeably without repeat at major clinical decision levels.

Keywords: Bland-Altman agreement, Harmonisation, Instrument comparison

INTRODUCTION

The clinical biochemistry laboratory as part of medical diagnostic system, routinely deals with the largest volume of samples with different varieties of tests lists. Different types of instruments are available in laboratory to carry out that test. Quality management system is required for both financial and safety purposes in clinical laboratory. It helps with medical decision-making and therapeutic procedures [1]. Considering the relevance of the obtained results, it has been increasingly necessary to have time and quality bound analyses performed and release the laboratory reports.

As advance in technology, many instruments with wide variety of principle or technologies like based on colorimetery, spectrophotometery, chemiluminescence, electrochemiluminescence principal are available in market and in laboratory to conduct sample processing in time bound period. Laboratories that have high test volume in their routines, generally end up having more than one biochemical analyser for sample processing, so as to optimise time of delivering the result.

To fulfill the laboratory quality goal, it is essential that result matching be obtained in all used biochemical analysers for examinations. Two or more instruments of the same principal, potency, model and manufacturer do not necessarily have similar work and performance. Documentary evidence is necessary with adequate statistical analyses to prove equivalence among all the tested instruments, based on the obtained results [2]. Discrepancies between the analysed values can be measured by various statistical methods like Deming regression and Passing Bablok, as well as Bland-Altman agreement analysis between methods [3,4]. Harmonisation of instruments are obligatory to ensure that different instruments can release equivalent laboratory results, thus establishing the laboratory quality standards and helps to achieve quality goal.

Continuously improved processes must be the main focus of any organisation to fully meeting client's needs and thus improving the level of competitiveness in the market [4].

Studies comparing a new instrument or method with an established method or instrument, to assess whether the new measurements are comparable with existing ones, frequently are conducted in clinical biochemistry laboratories [4,5]. Assessment of new instrument is based on applicability of new instrument or analytical performance of new instrument. Applicability includes cost of a new analyser; the costs, safety, and availability of reagents and calibration material; the space required by a new analyser; the requirements for sampling the material; the time to obtain a result when the analyser is ready and when the analyser is not ready; turn around time of analyser operator education; waste handling; etc. So, applicability of new instrument is totally subjective assessment. Analytical performance usually depends on statistical analyses and objective criteria of acceptability.

The aim of study was to assess equivalence and harmonisation in glucose results produce by ERBA XL-640 and ERBA XL-1000 in clinical biochemistry laboratory at SMIMER Medical College, Surat, Gujarat, India.

MATERIALS AND METHODS

A comparative study between two instruments was carried out in Clinical Biochemistry Laboratory, SMIMER Hospital, from June 2021 to October 2021. This study was carried out by comparison of results of glucose obtained in two automated systems ERBA XL-640 and ERBA XL-1000, based on protocol EP09-A3 of result harmonisation and review article-method comparison [6,7]. In this experiment, candidate instrument was ERBA XL-640 and comparative instrument was ERBA XL-1000 {Fully automated chemistry analyser (Transasia) in Clinical Biochemistry}.

Sample size calculation: As per CLSI EP09-A3 [6] protocol, minimum 40 serum samples should be analysed for two instrument comparison for any parameter. So, retrospectively 48 samples were selected from clinical biochemistry laboratory results data, obtained from Laboratory Information System (LIS), regardless of sex or age. Samples were selected in such a way that entire analytical range for glucose estimation gets covered.

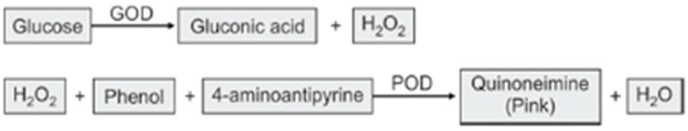
Procedure

No separate collection for study was performed. All samples sent by physician for some tests like liver functions test, renal functions test, Electrolytes were selected after completing all ordering tests. Identities of samples were masked. From low abnormal (less than 60 mg/dL) to high abnormal range (higher than 200 mg/dL), samples were selected for study. All sample selection criteria indicated by the protocol were observed, in relation to the good practices of sample handling, besides assessment of minimum sample volume and occurrence, major interference of lipemia, turbidity, haemolysis or icterus.

Once all 48 samples were identified, then proceeded with re-analysis by two instruments for comparison of glucose estimation.

Preparation of samples: All selected samples were subjected to repeat centrifugation for 5 minutes at 3000 rpm. Two separate set of serum aliquot of 200 µL were prepared for ERBA XL-640 and ERBA XL-1000.

Analysis of samples: Glucose protocols were generated in both instruments as per manufacturer's guideline. {Enzymatic, End point, GOD, POD; Sigma Diagnostics (India) Pvt., Ltd.,}



Glucose Test

Method: Glucose Oxidase-Peroxidase (End Point assay)

Manufacturer: Sigma Dignostic Pvt., Ltd.,

Principle: The enzyme glucose oxidase when reacts with glucose, water, and oxygen forms gluconic acid and hydrogen peroxide. The hydrogen peroxide can then be used to oxidise a chromogen or the consumption of oxygen measured to estimate the amount of glucose present.

System parameters (ERBA XL 640 and ERBA XL 1000):

Reaction Time: 8 minutes

Wavelength: 505 nm

Blank zero setting:Against reagent blank

Reaction Temperature: 37°C

Units: mg/dL

Sample volume: 2 microlitre

Reagent volume: 200 microlitre

Calibration of glucose methods are done with RANDOX Calibrator (CAL 1529) as per manufacturer's instruction. RANDOX QC (L2=1489, L3=1174) two levels run on both instruments every day to verify the quality of kit and instruments. To check the random errors on both instruments, samples with different ranges of glucose were analysed multiple times within run and between run for imprecision calculation.

Set the acceptable limit of difference between two instruments based on various criteria:

1. Based on Total allowable error as per described in CLIA criteria i.e., Target value ±6 mg/dL or ±10% (Whichever is greater) [8]
2. MEDx chart. (Method evaluation decision chart) [7]

Patient samples were measured by both instruments, preferably no more than four hours apart to avoid changes due to instability of the samples. To minimise systematic errors that might occur in only a single run, samples were assayed on several different analytical runs on five different days.

STATISTICAL ANALYSIS

The obtained results were evaluated in Microsoft excel and MedCalc free trial version software, by means of visual and quantitative analysis. In visual analysis, scattered graph and difference plot were used in form of Bland-Altman plot. Bland-Altman plot is a plot of difference against the average results of the method, which provides information on the relation between difference and concentration, which is useful to evaluate whether problems exist at certain ranges came by non linearity of one of the methods [9]. For quantitative analysis, bias was calculated from difference plot and regression analysis. The bias at medical decision levels with acceptable limits as described were calculated. If difference/bias is within acceptable criteria, correlation was classified as adequate at that medical decision levels.

RESULTS

All 48 samples were run within batch and between batches for calculating Standard Deviation (SD) and Coefficient of Variation (CV=(SD*100)/Mean). CV for each level for within batch and between batches was calculated. Maximum CV for one instrument was taken from all calculated CV. [Table/Fig-1] shows mean, SD and CV of within batch and between batches run in both the instruments. Highest CV was observed in the between batches run of 120 mg% sample (Level 1) on ERBA XL-640 and ERBA XL-1000 were 3.4 and 2.96, respectively.

ERBA XL-640					
	Within batch			Between batches	
Analysis of data	Level 1 (60 mg%)	Level 2 (230 mg%)	Level 3 (430 mg%)	Level 1 (120 mg%)	Level 2 (300 mg%)
Mean	68.4	244.4	449.7	117.6	305.6
SD	0.51	1.83	3.05	4	10.27
CV	0.75	0.75	0.68	3.4	3.36
ERBA XL-1000					
	Within batch			Between batches	
Analysis of data	Level 1 (60 mg%)	Level 2 (230 mg%)	Level 3 (430 mg%)	Level 1 (120 mg%)	Level 2 (300 mg%)
Mean	61.9	230.2	431.7	118.55	305.88
SD	0.56	2.65	3.59	3.5	7.7
CV	0.92	1.15	0.83	2.96	2.52

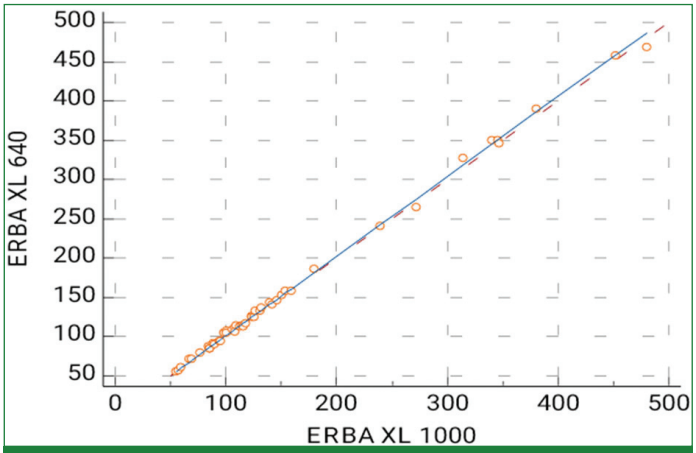
[Table/Fig-1]: Calculating CV % for within batch and between batches at different levels of glucose for ERBA XL-640 and ERBA XL 1000.
SD: Standard deviation; CV: Coefficient of variance

Acceptable limit of difference between two instruments based on Total Allowable error for glucose {(CLIA'88) [10] was 10% or 6 mg% (whichever is greater). The allowable limit of difference between the two instruments based on inherent imprecision (CV%) of both instruments is depicted in [Table/Fig-2]. For result analysis, 48 glucose samples those were selected from LIS based on their previous analysed glucose results covering analytical range were re-analysed in two different instruments on five different days for single time to harmonise both instrument for glucose parameter.

Maximum CV% of ERBA XL-640 for glucose	Maximum CV% of ERBA XL-1000 for glucose	Combined CV% of both instruments $\sqrt{CV640^2+CV1000^2}$	Allowable bias=0±(2×CV %)
3.4	2.96	4.50	±9.00

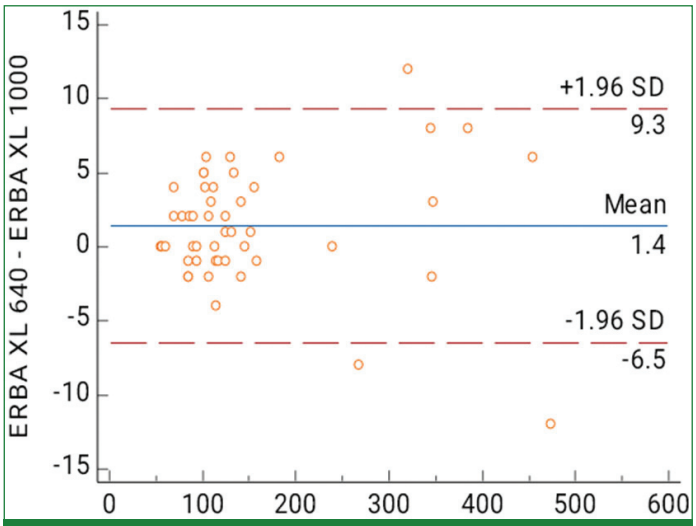
[Table/Fig-2]: Allowable limit of difference between two instruments based on inherent imprecision (CV%) of both instruments.

Results were plotted for visual inspection of correlation between two instruments by scatter graph and difference plot [Table/Fig-3]. Scatter chart for glucose comparison on both instruments. The two lines represent the line of equality (y=x) and the trend line. Data were linear and distributed around line of equality over all analytical range. Outlier is not visually detected in plot.



[Table/Fig-3]: Scatter plot representing serum glucose measured using ERBA XL-640 and ERBA XL-1000 instruments (n=48).
Orange dotted line is line of equality (y=x); Blue line is trend line

Difference plot with difference between two instruments (Y-axis) plotted for glucose against mean value of glucose by both instrument (X-axis) [Table/Fig-4]. The solid horizontal line represents the overall mean of the differences (bias=1.40 mg/dL with 95% confidence interval of 0.22 to 2.56), and the dashed lines show the range containing the mean of the differences±1.96 standard deviations, which represent the limits of agreement -6.51 mg/dL and 9.30 mg/dL).



[Table/Fig-4]: Absolute difference Bland-Altman plot for method comparison.
Solid horizontal line represents the overall mean of the differences; the dashed lines shows the range containing the mean of the differences±1.96 standard deviations

Percent Difference plot {(XL-640-XL-1000)*100/Mean} between two instruments (Y-axis) plotted for glucose against mean value of glucose by both instrument (X-axis) [Table/Fig-5]. The solid horizontal line represents the overall mean of the percent differences (bias% = 1.1 % with 95% confidence interval of 0.37 to 1.74), and the dashed lines show the range containing the mean of the percent differences ±1.96 standard deviations, which represent the limits of agreement -3.56% and 5.69%).

Difference plot with two orange lines representing 0±2*CV at mean of both instruments (allowable bias as per [Table/Fig-6]). Most of all data of difference are within the allowable bias range over all measurement ranges between instruments [Table/Fig-6].

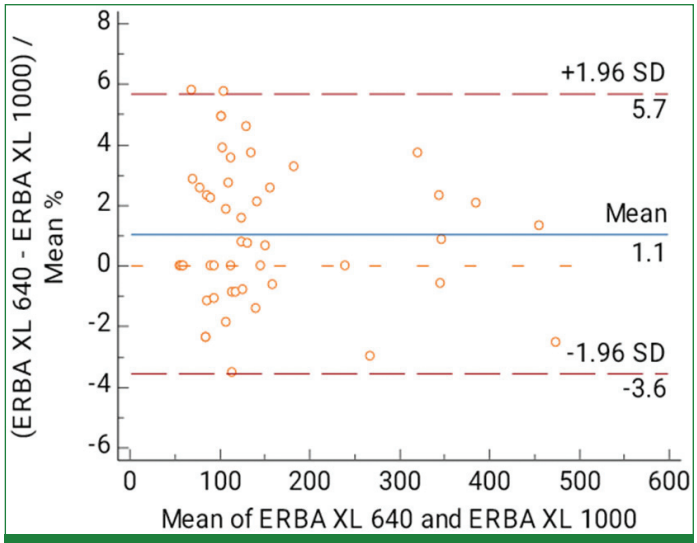
This is estimated as follows:

At 50 mg/dL orange line at {0±2*(50*4.5%)}={0±2*(2.25)}=0±4.5

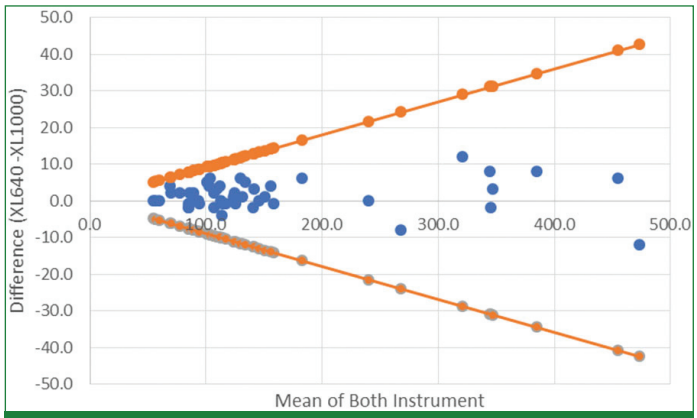
At 150 mg/dL orange line at {0±2*(150*4.5%)}={0±2*(6.75)}=0±13.5

At 250 mg/dL orange line at {0±2*(250*4.5%)}={0±2*(11.25)}=0±22.5

Quantitative estimation of bias: Linear regression of the glucose analyse by ERBA XL-640 and ERBA XL-1000 in patient samples, n=48; concentration range= 55-480 mg/dL [Table/Fig-7]. The solid

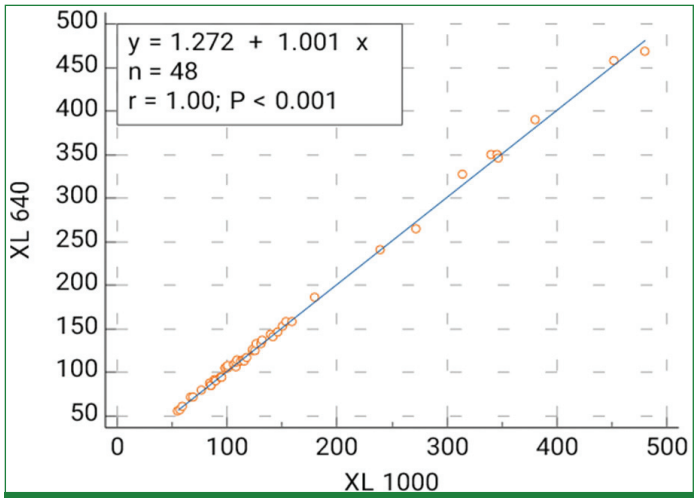


[Table/Fig-5]: Percent difference Bland-Altman plot for method comparison.
Solid horizontal line represents the overall mean of the differences; the dashed lines shows the range containing the mean of the differences±1.96 standard deviations



[Table/Fig-6]: Bland-Altman plot for method comparison (Difference plot for values with allowable bias as per inherent imprecision of both instruments.
Difference plot with two orange lines representing 0±2*CV at mean of both instruments)

line represents the regression equation (y=ax+b; where b-regression line's intercept and a- regression line's slope). The thin solid line represents the identity line consistent with correlation between the two methods (Correlation coefficient=1; p<0.001).



[Table/Fig-7]: Linear regression analysis for quantitative assay of bias.

In this study, the easiest way to judge acceptability based on preset analytical quality specifications is to use the maximum allowable total error (TEmax) and a method evaluation chart (MEDx chart), which is a graphical tool for comparing inaccuracy and imprecision and which has an analytical quality requirement stated in the form of allowable total error. In the MEDx chart, total allowable inaccuracy is on the y-axis and total allowable imprecision on the x-axis. Four

lines are drawn, each corresponding to the suggested criteria for TEmax as describe below:

A: {TEmax=Bias+1.65*Imprecision (CV)} from TEmax on Y-axis to (TEmax/1.65) on X-axis

B: {TEmax=Bias+2*Imprecision (CV)} From TEmax on Y-axis to (TEmax/2) on X-axis

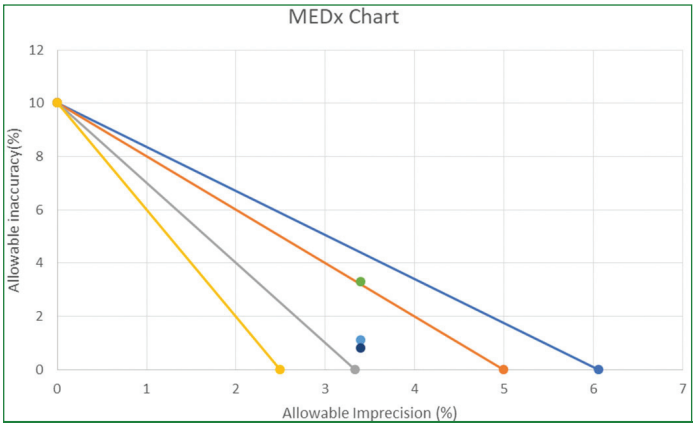
C: {TEmax=Bias+3*Imprecision (CV)} From TEmax on Y-axis to (TEmax/3) on X-axis

D: {TEmax=Bias+4*Imprecision (CV)} From TEmax on Y-axis to (TEmax/4) on X-axis

The three dot represents estimated impression (3.4%) and inaccuracy (3.3%, 1.1%, and 0.8% at medical decision levels 40 mg/dL, 120 mg/dL, 180 mg/dL, respectively) of the new method [Table/Fig-8,9].

Medical decision level	Calculated value for ERBA XL-640 from regression analysis	Bias in glucose assay for ERBA XL-640	Difference is acceptable or not as per acceptable limit [8]
40 mg/dL	41.3	3.3%	Acceptable
120 mg/dL	121.4	1.1%	Acceptable
180 mg/dL	181.4	0.8%	Acceptable

[Table/Fig-8]: Bias estimation at medical decision points and comparing with acceptable criterions.
Medical decision level are taken from westgard website "https://www.westgard.com/decision.htm"



[Table/Fig-9]: Linear regression analysis for quantitative assay of bias.
A: {TEmax=Bias + 1.65*Imprecision (CV)} from TEmax on Y-axis to (TEmax/1.65) on X-axis; B: {TEmax=Bias + 2*Imprecision (CV)} From TEmax on Y-axis to (TEmax/2) on X-axis; C: {TEmax=Bias + 3*Imprecision (CV)} From TEmax on Y-axis to (TEmax/3) on X-axis; D: {TEmax=Bias+4*Imprecision (CV)} From TEmax on Y-axis to (TEmax/4) on X-axis
Blue line- A; Orange line- B; Grey line- C; Yellow line- D

DISCUSSION

The use of different instruments in the laboratory is required for better equivalence between instruments in laboratory to provide same results in case of breakdown of one. Better equivalence can be achieved by using same manufacturer, same reagents, same QC materials and participating in same External Quality Assurance Services (EQAS) program in order to eliminate the largest number of variables. In the present study, both instruments from same manufacturer (Transasia) using same types of reagents, calibrators were used.

On comparisons of glucose end point measurements for two instruments, a strong correlation was obtained between the two instruments (r>0.995). Glucose values are linear and distributed around line of identity over the all range measured by two instruments [11]. Initial visual assessment of relationship between data produce by two instruments are adequate and correlate with each other. Correlation coefficient is not used as a measure of acceptability because it does not assess agreement, but assess association. Use of additional statistical test for further confirmation was required.

Difference plot provide the information on relation between difference and concentration, which may be helpful to evaluate the problems. It is also important to note that difference may be changed according to concentration change or independent of concentrations to rule out whether systematic error was constant or proportional to concentrations. Mean of two measurements were taken on X-axis because set of values from ERBA XL-1000 is not random error free. If we used ERBA XL-1000 values on X-axis for difference plot, it may introduce artificial error. In this case, majority of difference is plotted within the 1.96 SD of the mean value showing good agreement between the two instruments. Difference constant over horizontal axis can be suggestive of constant systematic error.

Percent difference plot denote the majority of percent difference is plotted within the 1.96 SD of the mean value showing good agreement between the two instruments. Percent difference is decreasing over horizontal axis suggestive of constant systematic error.

Differences between two instruments are within acceptable ranges and its suggestive of comparison is adequate. Quantitative analysis of difference is done and it is acceptable at medical decision level 40 mg/dL, 120 mg/dL and 180 mg/dL with compared to total allowable error given in CLIA'88 [10]. From MEDx (Method evaluation chart) chart two instruments for glucose estimation are identical within pre-set analytical quality specifications [10].

In previous study, [5] Labmax 240® and Labmax 240 Premium® from same company with different model were compared for various parameters and among them, one was glucose. The findings of this study were comparable to the present study.

The CLSI EP09-A3 document [3] denotes a strict protocol for comparisons between methods and bias estimate with the use of samples. It was used for the comparison of two methods that have similar measurement units. It requires a comparison with more than 40 samples in duplicate, at an interval of up to two hours between them. Good quality of the study of method comparison assumes adequately measured samples, with good result distribution, and values within the analytical interval of measures. It is important to observe that the equivalence study performed is used only in the range of analysed values, as numeric data of comparison (linear regression) cannot be extrapolated to concentration values outside the used range [12].

To decrease the frequency of test repetitions, reordering, recollection and erroneous results, harmonisation between the results obtained by different instruments is must. In addition to this harmonisation along with other analytical control practices such as internal and external controls bring safety and reliability to the laboratory. This also fulfills legal requirements. Using automated instruments compared to non automated, it's cost effective and reduce turnaround time. If any test equivalence fail between instruments, then it should be kept in mind during interpretation of result near clinical decision point and should be communicated to end user if required or should be re-verified by another method.

Limitation(s)

This study was conducted for comparison of glucose results in only two instruments. There is a scope for including multiple instruments from different manufacturers and multiple parameters.

CONCLUSION(S)

This study data denote the comparison of ERBA XL-640 and ERBA XL-1000 for glucose examination by end point method (GOD-POD) is acceptable and can be used interchangeably without repeat at major clinical decision levels. Harmonisation is important for reliability of results released by laboratory if more than one instruments are used by laboratory for same type of examinations.

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- iThenticate Software: Jan 24, 2022 (24%)

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