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Assessment of Pipetting Errors and its Contribution in Analytical Performance in A Tertyiary Care Hospital

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Abstract: *Clinical laboratory plays a very important role in diagnosis and treatment decisions and became an integral part of clinical medicine. So any error in testing will contribute to major mishap in clinical decision making. In our study we have checked for the errors in analytical part of our laboratory mainly focusing on the pipetting by analyzing routine tests like glucose, creatinine and total serum bilirubin. For each parameter, 7 samples were analyzed by 6 technicians out of which one sample is a standard material of known value and one of the technicians was a senior one. Mean, SD values calculated and checked whether obtained results were matching to CLIA acceptable performance criteria. Our results show the importance of technical skill mainly pipetting in analytical performance and how it can affect the final test results. It is emphasized that to reduce manual errors in pipetting, technicians working skills are to be updated and more of automation is preferred in the labs.*

Keywords: *Pipetting; Clia; Gtt; Glucose; Creatinine; Bilirubin*

I. Introduction

Medical laboratory plays a central role in the delivery of diagnostic services as over 70% of clinical decisions are taken based on laboratory reports (1). Two major types of errors may occur in a laboratory: Random errors that arise due to inadequate control on pre-analytical variables, patient identity, sample labeling, sample collection, handling and transport, measuring devices etc. Systemic errors that occur due to inadequate control on analytical variables; e.g. due to error in calibration, impure calibration material, unstable/ deteriorated calibrators, unstable reagent blanks etc. There has been a steady improvement in the quality of tests due to improved technology. As the present trend is more of evidence based medicine clinicians will rely on laboratory results for making diagnosis. This has led to the automation in clinical laboratory to decrease the turnaround time. Automation in clinical laboratory is a process by which analytical instruments perform many tests with the least involvement of an analyst. In fully automated machines, analysis was carried out with any number of selected tests on each sample. In Semi-auto analyzers, the samples and reagents are mixed and read manually (2).

In developing country like India most of the biochemical labs are still dependent on the manual methods/ semi auto analyzers rather than on auto-analyzers. Most of the manual experiments performed using spectrophotometer/colorimeter, and pipettes play a major role in performing a test. Air-displacement pipettes are used to perform so many analytical methods that they are often taken for granted. Pipettes are complex precision instruments subject to error due to mechanical failure and improper operator technique. Pipettes may contribute more inaccuracy and imprecision to laboratory results than any other single source (3).

As ours is a tertiary care center with majority of the sample load coming from pediatric population, pipetting errors contribute to variations in the test results thus affecting the treatment decisions. The effect of pipetting errors on clinical decision level values and how they affect the decision making and treatment of pediatric and antenatal patients is very important. Hence, the present study was undertaken to study the possible diagnostic error (pipetting error) by clinical laboratory technicians by testing routine biochemical parameters which are commonly done like blood glucose, serum Creatinine and total serum bilirubin.

II. Marerials And Methods

Fresh blood samples that arrived to our laboratory, daily for the analysis were used. Two ml samples were taken and centrifuged at 3000rpm to separate serum or plasma. Serum is used for the analysis of Total Bilirubin and Creatinine and plasma is for estimation of the glucose. The samples for analysis of glucose and creatinine were either random blood samples or two hour post glucose ingestion sample for GTT (glucose tolerance test) from the antenatal patients and for total bilirubin samples were from the pediatric and neonatal age group. The same sample was analyzed by different technical staff to check the variability in the test result.

III. Methodology

All the kits for the estimation were obtained from Erba Transasia® clinical diagnostics as a routine in our lab and instrumentation is semi-auto analyzers by Erba. Glucose is estimated by enzymatic GOD-POD method, total serum bilirubin by diazo method and serum Creatinine by modified Jaffe's method.

Total of 21 samples were analyzed separately for plasma glucose, total serum bilirubin and serum creatinine; 7 samples for each test parameter including one standard on a semi auto analyzer. Estimations done by a senior technician, as an unknown sample (sample 1) to avoid the bias was taken as reference and its values noted. Later all the samples were retested by the 5 different members in the lab represented as A-E to check the person to person variation by following the same procedure.

IV. Results

Glucose:

For plasma glucose estimation 2.0 ml random blood was drawn and analyzed for its glucose content. The recommended sample volume for the test is 10 µl. Glucose standard with the concentration of 100mg/d is taken as sample 1, it is analyzed by senior most technician and value noted. The glucose values analyzed by senior most technician and by other technicians were given in the table 1. According to CLIA (Clinical Laboratory Improvement Amendments)

criteria for acceptable performance for glucose is Target value ± 6 mg/dL or ± 10% whichever is greater. So the mean and SD for the experimental values were calculated and compared according to CLIA guidelines with obtained values.

Table 1. Plasma Glucose values for all the samples and their mean and 1SD

	Sample1 (mg/dl)	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Senior technician	100	291	98	105	94	101	101
Techn A	108	275	135	156	125	178	133
Techn B	106	404	106	90	75	55	83
Techn C	115	275	100	88	90	83	91
Techn D (mg/dl)	107	290	110	99	87	110	93
Techn E	111	265	94	89	110	91	128
Mean±SD	**109.4 ± 3.65**	**270.8 ± 104.88**	**109 ± 15.74**	**104.4 ± 29.18**	**97.4 ± 19.90**	**103.4 ± 46.15**	**105.6± 23.10**

According to CLIA guidelines acceptable performance for each sample noted. Performance in the lab for the sample 1 is within acceptable limits but for other samples the SD is > 10% compared to value of senior technician which is taken as standard value. There are some values which lead to misdiagnosis of the patient.

Serum Creatinine:

Creatinine standard with the concentration of 2 mg/dl is taken as sample 1 and is analyzed by the senior technician. **The recommended sample volume for the test is 50 µl** The values for creatinine are shown in table.2 along with its mean and 1SD. According to CLIA criteria for acceptable performance for creatinine is Target value ± 3 mg/dL or ± 15% whichever is greater. So the mean and SD for the experimental values were calculated and compared according to CLIA guidelines with obtained values.

Table 2. Creatinine values for all the samples and their mean and 1 SD

	Sample1 (mg/dl)	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Senior technician	2	0.80	0.80	0.60	0.80	0.7	0.90
A	1.92	0.91	1.0	0.70	0.86	0.86	0.89
B	2.02	0.95	1.0	0.70	0.85	0.84	0.93
C	1.86	0.90	0.93	0.73	0.92	0.78	0.94
D	1.95	0.99	0.97	0.75	0.95	0.83	0.96
E	2.06	0.92	0.93	0.67	0.83	0.77	0.88
Mean± 1SD	**1.96 ± 0.079**	**0.93 ± 0.036**	**0.97 ± 0.035**	**0.71 ± 0.031**	**0.88 ± 0.051**	**0.82 ± 0.039**	**0.92 ± 0.034**

According to CLIA difference of ±15% or ± 3mg/dl whichever is greater is taken to check the quality. Our creatinine results are well within these limits indicating good performance characteristics.

Total Serum Bilirubin:

Total serum bilirubin was estimated in pediatric and neonatal patients by taking 2.0 ml of serum. Sample volume required to perform the test is 25 µl. Similar to other 2 parameters standard with 2 mg/dl is taken as sample 1 and was analyzed by the senior technician. All the values for total serum bilirubin are shown in table.3 along with its mean and S D. According to CLIA criteria for acceptable performance for total serum

bilirubin is Target value ± 0.4 mg/dL or ± 20% whichever is greater. So the mean and SD for the experimental values were calculated and compared according to CLIA guidelines with obtained values.

Table 3. Total serum bilirubin values for all the samples and their mean and 1S D

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Senior technician	2.00	6.0	5.6	12.0	9.2	6.2	13.4
A	1.94	6.05	5.67	11.83	9.17	5.61	13.99
B	2.16	6.29	5.54	12.18	9.04	6.00	12.16
C	1.99	6.09	5.70	11.92	8.73	5.61	13.39
D	2.05	6.13	5.47	11.68	11.72	6.16	13.55
E	1.95	6.33	5.85	12.54	9.26	5.61	11.35
Mean± 1SD (mg/dl)	**2.04 ± 0.091**	**6.18 ± 0.125**	**5.65 ± 0.148**	**12.03 ± 0.338**	**9.58 ± 1.211**	**5.80 ± 0.264**	**12.89 ± 1.095**

According to CLIA guidelines acceptable performance for each sample noted. Variation of ± 20% for each sample calculated. They are 0.4, 1.2, 1.12, 2.4, 1.84, 1.24 and 2.68 mg/dl respectively. According to CLIA difference of ± 20% or ± 0.4mg/dl whichever is greater is taken to check the quality. When we observe individually there are 3 values which are out of CLIA quality rules and but doesn't lead to misdiagnosis as they are well within the normal range.

V. Discussion

Laboratory is a key partner in patient health safety so their reliability and quality of test is the area of main focus. Interpretation of laboratory tests is only one component of clinical decision-making, but it is an important and widespread one. Use of test results in clinical practice differs from use in research for three major reasons: clinical decision-making is always an exercise in classification; the unit of analysis is always an individual, never a group; and the decisions made have implications for that individual's well-being, which means that rare sources of error that have nothing to do with statistical distributions need to be considered (4).

So in this study we checked our laboratory performance and how it effects the outcome of a test result when it is performed by different individuals focusing mainly on pipetting errors; finally what is the impact on the outcome of the result in clinical decision making by performing experiments on 3 routinely tested parameters like glucose, creatinine and total serum bilirubin. Our results shown that there is discrepancy in the measurement of plasma glucose when tested by different individuals and some of the values were outside the clinical decision making limits resulting in the false diagnosis especially when the glucose was done for GTT. As we have taken care to maintain all other parameters standard in testing except pipetting by different individuals it shows the importance of pipetting errors in the analytical testing. In testing of total serum bilirubin there are a few values which couldn't satisfy the CLIA guidelines but were not affecting the clinical decision. Whereas in testing the serum creatinine we haven't found much discrepancy and values were within the CLIA analytical quality limits. For serum creatinine and bilirubin estimation the sample volume required is more as compared to the glucose and their reference range is also of different order. So the difference in each microliter of sample will affect the test result differently as we have observed with glucose in our results. This affirms the need for accurate pipetting and highlights the importance of pipetting in analytical testing.

VI. Conclusion

In this era of automation technology, clinical laboratories must abandon the manual methods and focus on strict compliance with quality procedures to enhance the reliability of the report generated from the lab so that the analytical errors are reduced to almost zero. Technician skills as well as calibration of pipettes will assure the most accurate results of the parameters hence the patients are saved from under diagnosis or over diagnosis hence appropriately managed to restore the health.

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