Research Article

Effect of Pipette Calibration on Quality Control Results in Khartoum State 2014

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Abstract: Pipette calibration significantly impacts the quality of liquid delivery in the clinical laboratory ,the aim of this study to determine that the effect of automatic pipette calibration on quality control results ,the experimental study involved 10 automatic pipettes 5 pipettes are with volume 1.0 ml and another 5 are with volume 0.1 ml ,all pipettes were examined for work calibration the precision and accuracy of automatic pipettes were estimated by using DW weighting method recommended by WHO according to ISO 8655.DIN 12650 after that precision and accuracy for urea, uric acid, and glucose were estimated in normal control sera by using CLSI document EP05.A2 describe the procedure for determining the precision pre and post calibration, the study found that a high percentage of pipettes performed outside. Established specifications for precision, accuracy and need recalibration .inaccuracy and imprecision of automatic pipettes had high effect on results of normal control the most important aspect of pipette quality control is a calibration and all pipettes must be periodically checked and also all new automatic pipettes must be verified and recalibrated .

Keywords: Pipettes calibration, precision, accuracy, Sudan

INTRODUCTION

An accurate pipette is one of the most important tools in performing accurate analytical work. an inaccurate or imprecise pipette will invariable lead to poor analytical work product ,regard less of how much care and skill is used performing the procedure .with use (and abuse), pipettes will become worn and less reliable .to maintain a valid work product ,the state of all pipettes must be periodically checked and verified [1].

Pipettes are subject to silent and random failures and have a higher rate of failure than many other laboratory instruments, the most important aspect of pipette quality control is a calibration frequency that ensures sufficiently high reliability. All Finn pipettes are factory calibrated and adjusted to give the volumes as specified with water. During factory calibration, performance is checked with five weightings at both the minimum and maximum volumes of the volume range. A calibration report is included with every pipette. Normally, the pipettes do not need adjustment, but they are constructed to permit readjustment for liquids of different temperature and viscosity [2].

Calibration is an operation to determine the actual volume delivered by a pipette together with an uncertainly associated with that volume [3].

Under a constant temperature and atmospheric pressure, the density of distilled water is constant. The volume of water can be determined by weighting dispensed water. The calibration of pipette is carried out by gravimetric method. When determining the volume of water, the accuracy of measurements is affected by ambient temperature, atmospheric pressure and relative humidity. These factors are usually combined to give the Z factor, used in calculation of volume of water. Then the calculated volume of water is compared with the theoretical volume to determine the accuracy and precision of the pipette [4]. The conversion factor Z is for calculating the density of water suspended in air at a test temperature and pressure.

Value of the conversion factor Z ($\mu l/mg$), as a function of temperature and pressure for distilled water is 1.0028

Error-free pipetting requires both precision and accuracy. A number of factors can affect these specifications. These form the main quantitative parameters for evaluating pipette performance [2].

Accurate, but not precise: The mean volume is the correct (set) volume, but the separate pipettings differ from the set volume. Precise, but not accurate: There is no variation between the separate pipettings, but the mean volume differs from the set volume. Accurate and precise: the mean volume is the set volume and there is no variation between the different

pipettings. Many factors affecting the accuracy of air displacement pipettes include Temperature**:** Temperature has many effects on pipetting accuracy. The factor that has the greatest effect is the temperature difference between the used delivery device and liquid. Density: the density (Mass/Volume ratio) affects the liquid.

Altitude: The geographic altitude affects the accuracy through air pressure [2].

MATERIALS AND METHODS

This is experimental study by collecting automatic micro pipette from five health centers in Khartoum state five with volume 1000 micro liter and another five with volume 100 micro liter and normal control sera was used and glucose ,blood urea ,and serum uric acid parameters were estimated in it by using CLSI document EP05. A2 describe the procedure for determining the precision of pre and post calibration. the study was conducted in Al-Neelain university faculty of medical laboratory sciences department of clinical chemistry from November to December 2014 ,admission was obtained from all health centers and approval was obtained from faculty of medical laboratory sciences and all five health centers .data were collected analyzed by using the Statistical Package for Social Sciences (SPSS version 16.00).

Method of pipette calibration D.W weighting method recommended by WHO according to ISO 8655.DIN 12650. This method was estimated precision and accuracy of automatic pipette.

Procedure to check calibration: The pipette was checked with the maximum volume (nominal volume), the minimum volume or 10% of the maximum volume, whichever is higher. For example, Finn pipette 0.5-10µl is tested at 10µl and 1µl. A new tip is first pre-wetted 3-5 times and a series of ten pipettings is performed with both volumes. A pipette is always adjusted for delivery of the selected volume. If the calculated results are within the selected limits, the adjustment of the pipette is correct.

Start work by using method of calibration DW weighting method recommended by WHO according to ISO 8655.DIN 12650 [3]:
➢ The precision and accuracy was estimated for all automatic pipettes pre and post calibration
➢ The precession and accuracy for glucose, urea and uric acid were estimated in normal control sera by using CLSI document EP05.A2 method pre and post calibration.) the acceptable limits for accuracy standards (is equal 5 for 1000 µl and 0.5 for 100 µl) and precession standards (is equal 2 for 1000 µl and 0.2 for 100 µl) [13].
➢ If the pipette fails calibration the pipette should be removed from use until it is serviced and passes a recalibration.
➢ Recalibration procedure: Firstly the pipette volume was checked and observed increase or decrease. Then the calibration tool (key) was placed into the holes of the calibration adjustment lock. And adjustment knob was turned anti clock wise to decrease, and clock wise to increase the volume. The performance test (checking calibration) procedure was repeated until the pipetting results were corrected.

RESULT

All automatic pipettes in all health centers were found imprecise and inaccurate pre calibration, and the precision and accuracy more than precision and accuracy standards that shown in table1, And the pipette calibration fails. As shown in table 1, precision and accuracy of pipettes in all centers were acceptable.

Table-1: precision and accuracy for automatic pipette with volume (1000 micro litre and 100 micro litres) pre and post calibration

Health Centre Number	1000 µl				100 µl			
	Accuracy		Precession (std. deviation)		Accuracy		Precession (std. deviation)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	9.2*	3.8	18.2*	2.0	.55*	.5	1.27*	.2
2	5.2*	4.4	2.8*	1.9	.63*	.5	.85*	.2
3	5.7*	5.0	2.48*	1.97	.71*	.5	1.4*	.19
4	6.8*	4.82	5.3*	1.89	13.7*	.48	16.9*	.18
5	5.3*	4.99	9.4*	2.0	13.9*	.49	8.4*	.2

Keywords
(*): mean that there was a significant difference from standard precision and accuracy.
Pre: pre automatic pipette calibration.
Post: post automatic pipette calibration.

Precession and accuracy of uric acid, urea and glucose pre and post calibration in normal control sera compared with total allowable error (TAE) for each test in tables bellow.

Table-2: Precision and Accuracy of Serum Uric Acid pre and post calibration the total allowable error for uric acid is ±17%

Health Centre Number	Accuracy		Precession	
	Pre	Post	Pre	Post
1	37.3	1.6	.5592	.4745
2	25.4	1.6	.5869	.2010
3	16.9	1.7	.4524	.4261
4	45.7	8.4	.2761	.7244
5	55.9	3.4	.7051	.6008

Table-3: Precision and Accuracy of blood urea, pre and post calibration The total allowable error for urea ±9%

Health Centre Number	Accuracy		precession	
	Pre	Post	Pre	Post
1	30.3	11.1	1.2822	.8961
2	27.7	8.8	.7569	1.0679
3	18.8	4.6	2.3687	2.1039
4	26.1	8.5	.9846	1.5017
5	32.3	7.3	.8347	1.2760

Table-4: precision and accuracy of blood glucose pre and post calibrationThe total allowable error for glucose ±10%

Health Centre Number	Accuracy		precession	
	Pre	Post	Pre	Post
1	21.4	9.5	.8294	4.8759
2	20	3.1	1.2055	2.8539
3	18.3	5.2	1.3555	4.0528
4	22	3.6	1.6554	7.7048
5	21	13.1	3.8070	2.5105

DISCUSSION

Poor precision and accuracy affect the quality control results. After pipettes were collected from the health centers, precision and accuracy were calculated for each pipette, the accuracy, standard deviation and CV failed pre calibration and became less than standard value that shown above. After that serum uric acid, blood urea and glucose were estimated in normal control sera 20 times before and after calibration for precision and accuracy in these health centers that shown in table (2), (3) and (4), then compared with standard precision and total allowable error for each test and found that results were affected after calibration. And before calibration, the results were not reliable and silent failure occurred.

CONCLUSION AND RECOMMENDATION

In this study had been found that all pipettes from these health centers performed outside established specifications for precision and accuracy, yet the operators were unaware that silent failures were occurred and had not taken these mal functioning pipettes out of service. The study shown that Inn accuracy and imprecision of automatic pipette had high effect on quality control results. Since pipettes are subject to silent and random failures and have a higher rate of failure than many other laboratory instruments, the most important aspect of pipette quality control is a calibration frequency that ensures sufficiently high reliability. And the calibration must be done every three months and also verification for new automatic pipettes.

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