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Influence of Successive Manual Pipetting of Multiple Samples on Precision and Reliability of Glucose Test Results

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Abstract

Air-displacement micropipettes are precision equipment; however, they are affected by temperature change. The study investigated whether hand warmth generated during successive manually pipetting 10 µL in multiple samples using air-displacement micropipette can influence accuracy of glucose estimations. Venous blood sample (5 ml) was collected from an informed and a consented apparently healthy normoglycaemic adult (male) following ethical approval. One hundred replicates from aliquot plasma were divided into five ranges (R1-R5) of twenty replicates each for fasting plasma glucose (FPG) estimation. Gilson P20 air-displacement micropipette (Gilson, UK) was adjusted to pipette 10 µL manually under routine and repeatability conditions by an experienced analyst. Relative humidity, room temperature and analyst's palm temperature were recorded before and after successive manual pipetting. FPG level was determined using Glucose-oxidase (GOx) method, and intra-analyst coefficient of variation (CV) calculated. Data were analyzed using descriptive statistics and one-way analysis of variance; association among variables was determined by Pearson's correlation analysis, and significant level was set at $p < 0.05$. Duration of successive holding micropipette while manually pipetting 100 replicates in five ranges correlated positively with analyst's palm temperature ($r = 0.881$, $p = 0.048$). Replicates in range 5 (81-100) had a significantly decreased FPG level ($p = 0.000$) with intra-analyst CV of 8.8% compared to ranges. Results suggest that under routine laboratory and repeatability conditions, successive manual pipetting of 10 µL more than 640 seconds or 60 samples using air-displacement micropipette may result in significant volume errors with imprecise glucose estimates.

Keywords: Micropipette, hand warmth, accuracy, imprecision, glucose test

Introduction

Micropipettes are very important apparatus used in clinical analytical chemistry, biotechnology, forensic and serology laboratories. They are designed to easily and accurately aspirate and dispense set amount of sample, reagent or liquid in microlitres ranging from 1µl to 10000 µl (Krishnan *et al*., 2019). Micropipettes are classified based on operating principle, mechanism, and number of channels or volume. Air-displacement and positive displacement micropipettes operate on different principles. However, both can be operated manually or electronically. The single channel piston-operated air-displacement micropipettes are mostly used in clinical chemistry laboratories for routine analysis; they can aspirate and deliver set volume with high accuracy if calibrated in compliance with the International Organization for Standardization (ISO) 8655-6:2002 (ISO, 2023) and used as described in Eppendorf Userguide No 20 (Eppendorf, 2015). Accuracy of a diagnostic technique gives reliability to test results and is crucial in modern medicine (Dhamnetiya *et al.,*2021) in which about 70% medical decisions are based on laboratory findings. Thus, accurate micro-pipetting is imperative to preventing errors in test results

(Pandya *et al.,* 2010; Eppendorf, 2015). Moreover, quantitative evaluation of accuracy of test results is important to delineate the extent of self-confidence and the dependability of the medical decisions based on such laboratory test results (Menditto and Patriarca, 2007).

However, despite the low cost, easy use and minimal risk of contamination on repeated use of air-displacement micropipette, the typified captive air volume and the working principle render this type of pipette susceptible to environmental factors such as temperature, barometric pressure and humidity when compared to positive displacement micropipette (Millet and Barthlen, 2007; Feldmann and Lochner, 2016). Studies have shown that accuracy of pipetting improves at a steady temperature. But change in temperature between air-displacement micropipette and samples can result in wrong and inconsistent aspiration and dispensing of set volumes (Blue *et al.,* 2023).

Globally, more than half a billion people are living with diabetes mellitus (DM) and over the decades prevalence of DM has increased alarmingly affecting 537 million adults in 2021. Also, projections by the International Diabetes Federation (IDF) have shown that 634 million and 784 million people will be diabetic by 2030 and 2045 respectively (IDF, 2021). Unbiased and precise blood glucose estimates play a critical role for timely diagnosis and proper control of DM complications. Notably, nowadays, due to the soaring global increase in diabetes, especially in low- and middle-income countries. Some clinical chemistry laboratories can receive, for instance, one hundred or more blood samples in a day. In this scenario, it is obvious that in some laboratories that have not been fully automated to handle large sample sizes, analysts therefore, mechanically pipetted enormous blood samples in a succession with a prolonged pipetting duration. By principle, it is posited that warmth transfer from analysts' palm to air-displacement micropipette can influence dispensed volumes (Pushparaj, 2020; Carle *et al.,* 2023) while analytes are erroneously estimated. Cognizance of this influencing factor on the accuracy of glucose test results is important considering the small volume (10 µL) used and critical implications on medical decisions informed by such test results. However, the effects of hand warmth on precision under routine laboratory and repeatability conditions remain to be elucidated. This study investigated whether hand warmth generated during successive manually pipetting 10 µL in multiple samples using air-displacement micropipettes can influence accuracy of glucose test results.

Material and Methods

Materials: A Gilson micropipette (0-20 µL), ApTechDeals HTC-1 Digital Hydrometer Thermometer Humidity Meter, Timer and stopwatch (TFA: Kat Nr.38.2021), Mini LED Digital Temperature Meter Display Probe Digital Sensor LCD Thermometer for Refrigerator, Refrigeration Machine (ASHATA), Spectrophotometer, clean test tubes and new tips.

Chemical: Analytical grade glucose-oxidase reagents kit (Randox Laboratories Limited, Crumlin, and County Antrim, United Kingdom) was for glucose estimation.

Ethics statement: This study was conducted in accordance with the World Medical Association's Declaration of Helsinki (2013). Ethical approval was obtained from Bowen University Research Ethics Committee (BUTH/REC-817). The study participant was properly informed of the nature of the research and written informed consent obtained prior to participation in the study.

Study design: One participant was recruited for this study. The study was divided into five ranges designated as range 1 (R1), range 2 (R2), range 3 (R3), range 4 (R4) and range 5 (R5). Each range had 20 replicates from aliquot plasma. The replicate ranges were 1-20, 21- 40, 41- 60, 61-80, and 81-100 for R1, R2, R3, R4 and R5, respectively.

Pipetting procedure: Pipetting was done by a well-trained Medical Laboratory Scientist (analyst) with experience on optimum handling of manual pipettes as described by Eppendorf (2015). A well calibrated 20 µL pipette (Gilson, UK) adjusted to dispense 10 µL was used to pipette 100 replicates from aliquot plasma into clean test tubes in five ranges (R1-R5) of 20 replicates each.

Before pipetting, room temperature (RmT) and relative humidity (RH) were taken using ApTechDeals HTC-1 Digital Hydrometer Thermometer Humidity Meter. Analyst's (metacarpus) palm temperature (PmT) was recorded using temperature meter (Mini LED Digital Temperature Meter Display Probe Digital Sensor LCD Thermometer for Refrigerator). Glucose reagent, standard, internal quality control sample (control) and micropipette were kept on the laboratory workbench for one hour to attain the room temperature. Duration of successive hand holding of micropipette and pipetting (DSHP) and the corresponding PmT for R1, R2, R3, R4 and R5 were recorded using a timer and temperature meter, respectively. Quality control sample was pipetted after each 20^{th}, 40^{th}, 60^{th}, 80^{th} and 100^{th} replicate, and immediately after which RH, RmT, DSHP and PmT were recorded. PmT was determined by quickly holding the temperature sensor for 10 seconds (time allowed for a steady PmT reading). Manufacturer's recommended tips were used, and each dispense was performed with a new tip. Tips were immersed 2 mm below the meniscus in the sample and were pre-wetted two or three times before dispensing into the respective tubes. During aspiration of the sample, pipette was vertically held at a 90° angle and dispensed at a 45° angle against the wall of the test tubes with blow-out. Pipetting was done by forward mode. The entire pipetting duration was in a succession under routine laboratory and repeatability conditions.

Blood sample collection and determination of fasting plasma glucose (FPG): After an overnight fast, five milliliters (5ml) venous blood was collected from the participant aseptically by venipuncture using a 10 ml syringe and 25 G needle into a fluoride oxalate bottle. It was kept on a laboratory bench for 10 minutes and centrifuged at 3000rpm for 5 minutes. The plasma extracted was kept for 1 hour to attain the room temperature before glucose estimation using the Glucose-oxidase method described by **Trinder (1969)**. Reference range for FPG was 3.5-5.5 mmol/L.

Statistical analysis: Data was analyzed using the PASW Statistic 18, a statistical package formerly known as SPSS Statistics from SPSS Inc, (Chicago IL, United States of America) by one-way analysis of variance and Boneferroni post hoc test; p-value less than 0.05 ($p<0.05$) was considered statistically significant. Results were presented as mean ± standard deviation. Association between variables was done using Pearson's correlation analysis.

Results

Parametric values before and after successive manual pipetting: Table 1 shows values for the laboratory conditions (relative humidity (RH) and room temperature (RmT]), analyst's palm temperature (PmT) and duration of hand-holding micropipette (DHP) before and after successive manually pipetting of 100 replicates. The operated RmT and RH were within the acceptable laboratory temperature (15-30°C) and relative humility (>55%). After successive manually pipetting the first twentieth replicates in R1**,** PmT decreased by 0.5°C but progressively increased by 0.2°C, 0.3°C, 0.5°C and 0.9°C in R2, R3, R4 and R5, respectively. Successive pipetting from R1 to R5 for 682 seconds increased PmT by 0.9°C.

Tables 1: RH, RmT, PmT and DSHP befor e and after successive manual micro pipetting

Parameter	Before	After					Mean
		R1 (0-20)	R2 (21-40)	R3 (41-60)	R4 (61-80)	R5 (81-100)	
RH (%)	65	65	65	65	66	65	65±0.45
RmT (°C)	26.1	26.1	26.1	26.1	26.3	26.2	26.2±0.09
PmT (°C)	32.5	32.0	32.2	32.3	32.5	32.9	32.4±0.34
DSHP (S)	0	232	465	640	790	914	608±269.08

Table 2 presents values for control, fasting plasma glucose (FPG) and intra-analyst coefficient of variation (CV). There was a significant variation ($p < 0.05$) in FPG level among the five ranges. Post hoc analysis showed that R5 had significantly lowered FPG ($p = 0.000$) compared to other ranges and intra-analyst CV of 8.8%. The mean intra-analyst CV was 10.2% (range, 7.9% -15.8%).

Table 2: Control, FPG and CV i n five ranges of 20 replicates

Variable	Range of Replicates					Mean	F-value	P-value
	R1 (0-20)	R2 (21-40)	R3 (41-60)	R4 (61-80)	R5 (81-100)			
Control (mmol/L)	3.2	3.0	3.1	4.8	2.4	3.3±0.85		
FPG (mmol/L)	5.1±0.44	5.2±0.41	5.2±0.43	5.4±0.85	4.3±0.38*		13.064	0.000
CV (%)	8.6	7.9	8.3	15.7	8.8	10.2		

*Significant at $p < 0.05$

Correlation among variables: Table 3 shows a significant ($r = 0.881$, $p = 0.048$) positive correlation between DSHP and PmT. However, no association was found between DSHP and FPG, and CV.

Table 3: Correlation among DSHP, APmT, FPG and CV

Parameter	Index	r-value	p-value
DSHP (S)	PmT	0.881*	0.048
	FPG	--0.534	0.354
	CV	-0.067	0.915

Discussion

Inaccurate blood glucose results can lead to misdiagnosis, treatment errors (Erbach *et al.,* 2016) or death. This study investigated whether hand warmth generated during successive manually pipetting 10 µL in multiple samples using air-displacement micropipette under routine laboratory and repeatability conditions can affect accuracy of glucose test results. Trueness and precision are the qualitative performance characteristics that define accuracy. Accuracy is used to assess the influence of both systematic and random errors on analytical methods or measurement results (Chesher, 2008). While accuracy is defined as the closeness of agreement between the value of a measurement and the true value of the measured while trueness is the difference between the mean of numerous measurements on the same sample and its true value. Contextually, trueness indicates the effects of systematic error on the measurement results which are expressed quantitatively as bias. However, precision is the closeness of several repeated measurement values (Menditto *et al.,*2007). Therefore, in analytical methods, assessment of precision is a part of the process of validating a method for confirmation of its accuracy and suitability for use (Chesher, 2008). Precision informs analysts about the effects of random error on the measurement results which is quantitatively expressed as standard deviation of mean (±SD) or analytical coefficient of variation (CV). Air-displacement micropipettes are precision equipment which analytical performance is highly influenced by factors including temperature disequilibrium and hand warmth (Carle, 2008).

In this present study, the significantly lowered FPG level in R5 (81-100 replicates) compared to R1, R2, R3 and R4 (1-80 replicates) may be due to combinatory factors including increased warmth (0.9°C) transferred from the analyst's palm to the air-displacement micropipette, and the minute volume (10 µl) dispensed. Successive manually pipetting for more than 790 seconds increased the PmT from 32.0 °C to 32.9 °C. Following this warmth transfer to micropipette, it could be possible that a thermal

disequilibrium exists between the temperature of the micropipette and the attained room temperature (26.2°C) of the aliquot sample. For accurate pipetting and result, it is recommended that tips, micropipette and sample attained the same ambient temperature or within 0.5°C (ISO, 2023). Previous studies noted that extensive handholding of air-displacement micropipette has the tendency to expand the captive air resulting in smaller dispensed volumes particularly with minute volume (Carle, 2014; Pushparaj, 2020). This probable explained the reason for the significant decreased FPG observed in R5.

Moreover, the 0.5°C decreased in PmT in R1 is suggestive of initial warmth transferred from analyst's palm to the micropipette while pipetted the first 20 replicates. However, PmT was found to increase from 32.0°C to 32.9°C with successive pipetting, which accounts for the significant positive correlation between DSHP and PmT. Earlier study (**Lippi *et al*.,2017**) on manual pipetting using air-displacement micropipette showed a significant negative correlation between set volumes dispensed and mean intra-analyst pipetting imprecision (CV) of 5.2%; ranging from 0% to11% for 10 µL. On the contrary, we found mean intra-analyst pipetting CV of 9.9 %; ranging from 7.9% to15.8%. This could partly be caused by inconsistent over delivery of nominal volume in R4. This finding agrees with (Salas, 1995) who previously reported pseudo imprecision with increased mean value of an analyte.

Overall, our findings show a positive correlation between duration of successive handholding of micropipette and analyst's palm temperature during pipetting, and that prolonged successive manually pipetting 10 µL more than 640 seconds or 60 samples using air-displacement micropipette under routine and repeatability conditions may increase intra-analyst imprecision that can ultimately truncate accuracy and reliability of glucose test result.

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