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Original Research Article

ACCURACY OF FOUR HUMAN POINT-OF-CARE GLUCOMETERS FOR BLOOD GLUCOSE DETERMINATION IN RATS

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ABSTRACT

This study compared the accuracy and agreement of four commonly available point-of-care glucometers (PCGs) with a reference method for blood glucose determination in rats. Forty apparently healthy rats were used for the study. Blood samples were collected from the retro bulbar plexus using capillary tubes and the four PCGs were used to determine the blood glucose concentrations immediately. Thereafter, the samples were put into test tubes treated with anticoagulant. The samples were then centrifuged at 3,000 g for 5 minutes and plasma harvested. Blood glucose test kit was used to measure glucose concentrations in plasma by the glucose oxidase method. Data generated were analysed using Correlation coefficient, Student's t-test and Bland Altman Plot. Results showed that the mean values generated by Accu-Answer® and Fine test® PCGswere comparable with the values generated by the laboratory method, while that generated by On Call Plus II® and BG Check® PCGs varied significantly from the laboratory method. Therefore, Accu-Answer® and Fine test® PCGs may be used in rats for diagnostic or research purposes with reasonable accuracy while On Call Plus II® and BG Check® PCGs may be used with caution.

Keywords: blood glucose determination, diagnosis, point of care glucometer, rats, research

INTRODUCTION

Diabetes mellitus (DM) has become one of the most common chronic diseases in the world, with 422 million people affected worldwide (Marques *et al.,* 2020). In Africa, over 14 million people are currently affected with the number expected to double by 2040 (IDFA, 2015).

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia caused by lack of adequate insulin or insulin resistance. The persistent hyperglycemia results in microvascular complications mainly in the kidney, retina and nerves, causing morbidity and mortality (Sugimoto *et al*., 2003; Johansen *et al*., 2005).

Although natural cases of DM are low in rats (McCready *et al*., 2023), search for solution for DM in human has resulted in dozens of induced cases of DM (Akbarzadeh *et al*., 2007; Gheibi *et al*., 2017; Banda *et al*., 2018; Aba & Ede, 2019; Furman, 2021; Fajarwati *et al*., 2023ab).

Hypoglycemia induced by insulinoma had also been reported in rats (Adissu & Turner, 2010).

Point-of-care glucometers (PCGs) are commonly used in management of glucose disorders in both humans and animals and for biomedical research due to their rapid generation of results, cheapness, use of small sample quantity and need for little or no expertise compared to the conventional method (Lieske *et al*., 2002).

This has resulted in the use of PCGs for diagnostic and research purposes with little or no knowledge of the validation status, as well as little literature to support device accuracy (Morley *et al*., 2018; Okorie-Kanu *et al*., 2020).

Several studies have shown strong agreement in values obtained by human PCGs with laboratory methods in both companion and farm animals (Wess & Reusch, 2000ab; Johnson *et al*., 2009; Hackett & McCue, 2010; Fowler *et al*., 2011; Domori *et al*., 2014, Selleri *et al*., 2014; Mair *et al*., 2016; Morley *et al*., 2018; Okorie-Kanu *et al*., 2018ab, 2021, 2025). However, reports of overestimated results by some PCGs in several species have been a source of great concern for veterinarians (Hollis *et al*., 2008; Cohen *et al*., 2009; Cohen *et al*., 2010; Acierno *et al*., 2012; Burdick *et al*.,

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2012; Hornig *et al*., 2013; Petritz *et al*., 2013; Higbie *et al*., 2014; Summa *et al*., 2014; Kang *et al*., 2015; Clemmons *et al*., 2016; Mair *et al*., 2016; Togashi *et al*., 2016; Okorie-Kanu *et al*., 2018ab, 2021).Our earlier study in rats validated one PCG for use in rats and showed the other to be inappropriate (Okorie-Kanu *et al*., 2021). Considering how important rats are in biomedical research, this work was designed to validate more PCGs for use in glucose determination in rats.

MATERIALS & METHODS

Forty (40) apparently healthy male rats weighing between 120 and 180 grams were used for the study. They were kept for two weeks for acclimatization in the Research animal house of the Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, Abia State and feed and clean water given *ad libitum*. Random blood samples were collected from ten rats in a week for four weeks. The rats were individually restrained with minimal stress and 2 ml of blood was collected from the retro-bulbar plexus using a capillary tube. Blood glucose levels were assayed immediately after blood collection with the four PCGs.

The blood samples were put into clean test tubes treated with ethylene di-amine tetra-acetic acid (EDTA), then centrifuged at 3,000 g and plasma harvested.

All protocols in the experiment were approved by College of Veterinary Medicine Research Ethical Committee, Michael Okpara University of Agriculture, Umudike, with Ethical Approval Number (MOUAU/CVM/REC/202429).

POINT-OF-CARE GLUCOMETERS

Blood glucose levels were determined immediately with the four PCGs. The four PCGs were used to determine the blood glucose levels according to the instructions of the manufacturers. Test strips were inserted into the glucometer port for test strip and observed until a flashing blood image appeared on the glucometer screen. Thereafter, the test strip was placed on a drop of blood and the strip pad filled by capillary action.

The blood glucose concentration in mg/dl was displayed on the screen after 5 seconds in all methods. The range of glucose concentration detectable for Accu-Answer®, On Call Plus II®, Finetest® and BG Check® were 10 - 600 mg/dl, 20 - 600 mg/dl, 10 - 600 mg/dl and 20 - 600 mg/dl respectively. The principle enzymatic reaction of Accu-Answer®, On Call Plus II® and Finetest® is glucose oxidase and hexokinase dehydrogenase for BG Check® PCG.

Test strips of PCGs deliver results that correspond with the plasma glucose concentration (D'Orazio *et al*., 2005; Steffes and Sacks, 2005).

LABORATORY METHOD

Blood glucose level was determined using glucose test kit (Randox®) based on GOD POD method (Glucose oxidase method) according to Trinder (1969) for in-vitro determination of blood glucose in serum or plasma. The working reagent (1 ml) was mixed with 0.01 ml of serum sample and allowed to stand for 10 minutes at room temperature. A standard was prepared as described above. The absorbance of both the samples and standard were read against the contents of the blank at 505 nm using a Cole Parmer 1200 spectrophotometer (Cole-Parmer Instrument Co., USA). The glucose concentration was obtained with the formula below:

$$\text{Glucose Conc. } (\frac{\text{mg}}{\text{dl}}) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 100$$

The minimum detectable concentration of glucose with an acceptable level of precision is 6.18 mg/dl.

The data obtained were analysed using Correlation Coefficient Analysis, Student's t-test using IBM®SPSS® Statistics, Version 25 and Bland Altman Plot (Bland & Altman, 1986). Values of $P < 0.05$ were considered significant.

RESULTS

There were strong positive correlations between the four glucometers and the laboratory method (Table I). The values generated by Accu-Answer® and Fine test® PCGs were comparable ($p > 0.05$) with the laboratory method (Table I). On Call Plus II® PCG significantly underestimated the blood glucose values while BG Check® significantly overestimated the blood glucose values when compared with the reference method (Table I). Bland Altman tests showed close agreements between Accu-Answer® and Fine test® PCGs and the laboratory method (Figures I and III) and weak agreement between the On Call Plus II® and BG Check® and the laboratory method (Figures II and IV).

Figure I: Bland Altman Plot of Accu-Answer® PCG

TABLE I: BLOOD GLUCOSE CONCENTRATION (MG/DL) OF 40 RATS DETERMINED WITH DIFFERENT METHODS

Methods	Mean ± (SE)	Standard deviation	Min. value	Max. value	Coefficient
Accu-Answer®	85.00 ± 2.14	13.51	61.00	114.00	0.904
On Call Plus II®	77.88 ± 2.58*	16.34	47.00	119.00	0.870
Fine test®	89.35 ± 2.42	15.28	65.00	130.00	0.897
BG Check®	92.70 ± 2.77*	17.52	55.00	126.00	0.847
Laboratory method	84.83 ± 2.68	13.37	57.00	113.00	

***Significant difference between the methods and laboratory method ($p<0.05$)**

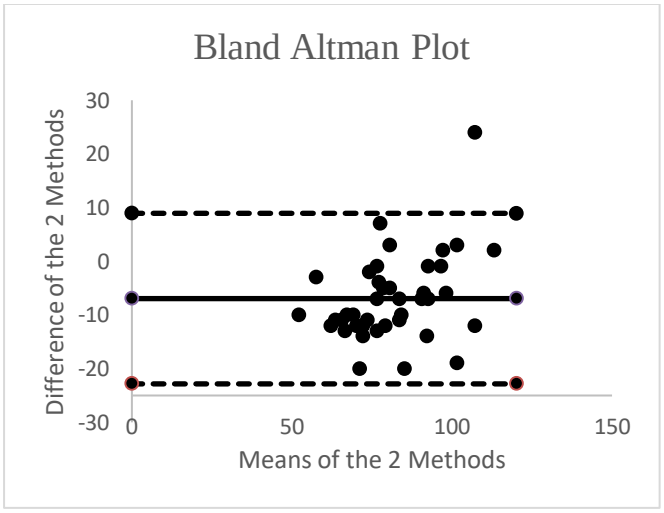

Figure II: Bland Altman Plot of On Call Plus II® PCG

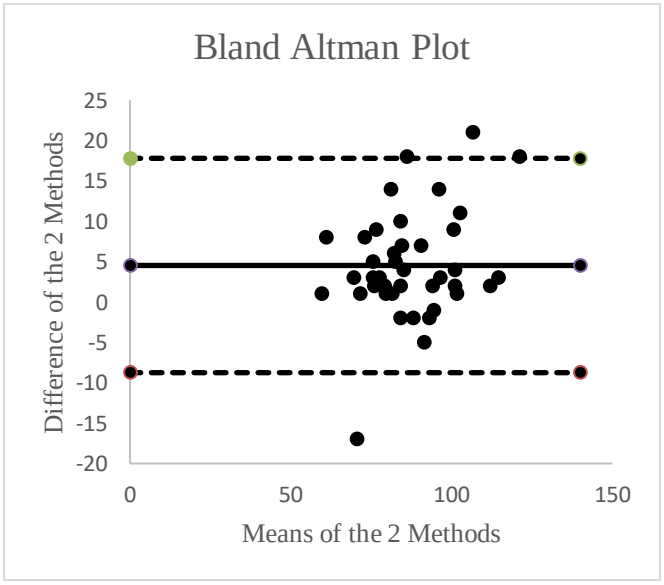

Figure III: Bland Altman Plot of Fine test® PCG

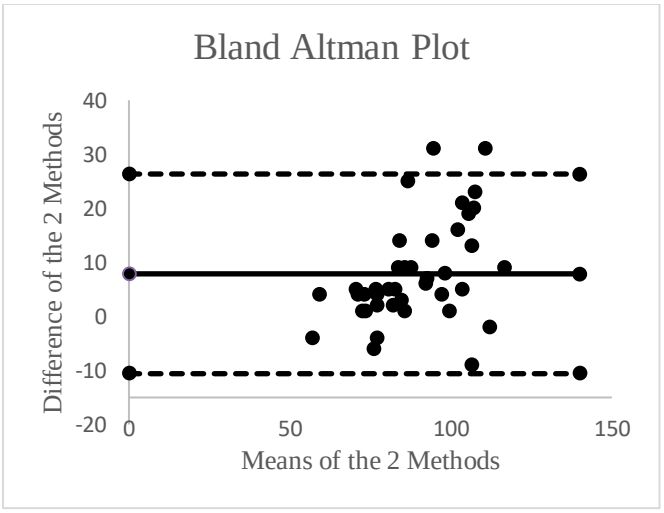

Figure IV: Bland Altman Plot of BG Check® PCG

DISCUSSION

Results of the present study indicate that Accu-Answer® and Fine test® PCGs agreed well with the laboratory method. The correlation coefficient and the result of Bland Altman Plot indicate strong agreement between them and the laboratory method. Therefore, Accu-Answer® and Fine test® PCGs can be used for blood glucose determination in rats with reasonable accuracy. However, although the values generated by On Call Plus II® and BG Check® PCGs varied significantly from the laboratory method, both can still be used to determine blood glucose without any negative clinical implication according to the guidelines of American Society for Veterinary Clinical Pathology (ASVCP) on the use of PCGs; as the values still fell within the 20% total allowable error margin (Gerber & Newman, 2016). Variations in size and shape of RBC have been attributed to the variations in blood glucose values generated by human PCGs in animals (Beemer *et al*., 2013).

Results of the present study have given more credence to the importance of validation of PCGs in specific animal species before use to avoid use of erroneous results in management

of glucose disorders in animals with the attendant adverse effects (Cohn *et al*., 2000).

CONCLUSION

Accu-Answer® and Fine test® PCGs may be used in rats for research purposes with reasonable accuracy while On Call Plus II® and BG Check® PCGs may be used with caution.

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