Evaluation of Performance Characteristics of an In-house Glucose Reagent Compared to Analyzer Specific Commercial Glucose Reagent

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Abstract - Blood glucose level is the most frequently analyzed parameter in a routine clinical laboratory in order to assess diabetes mellitus. Currently, commercial reagent kits with high costs are used for this test. However, the same reagent can be prepared in the laboratory at a lower cost. The aim of this study was to examine the performance of an in-house reagent method under standard laboratory conditions with the analyzer specific commercial glucose reagent. An evaluation study was carried out at the Clinical Pathology Laboratory, Teaching Hospital, Karapitiya using 200 randomly selected retained blood samples. Glucose values were determined by in-house glucose reagent and commercial glucose reagent. Correlation and the agreement between the two methods were determined. Accuracy, sensitivity, specificity, precision, and stability was checked for the in-house method. Daily IQC and monthly EQA samples were run to assure precision and accuracy. The results were significantly correlated (r=0.9993; p=0.001), and the two methods indicated a good agreement with a positive bias of 0.835±0.488 mg/dL in Bland Altman analysis. There was a good agreement between 0-300 mg/dL. At concentrations above 300 mg/dL, a tendency towards increasing scatter was observed, which could be due to the low number of sample size in this range. Accuracy, sensitivity, and specificity were 96.5%, 96.15% and 97.14% respectively. The in-house method was linear up to 1000mg/dL. An intra-assay precision (CV) of 6.88 and 2.38% and an inter -assay precision of 2.21 and 3.34% were obtained for normal and high levels of glucose respectively. The reagent was stable for a period of three months at 2-8˚C.The in-house glucose reagent is more cost-effective and possesses similar performance characteristics and good stability, compared to the analyzer specific glucose reagent. Thus, it can be adopted for analysis of plasma glucose in routine laboratory checkups.

Keywords: glucose, performance characteristics, correlation coefficient

I. INTRODUCTION

Glucose is the primary source of energy for the body. Mainly, the body obtains glucose through the digestion of sugar and starch in carbohydrates, in the fed state. In the fasting state, gluconeogenesis and glycogenolysis maintain glucose concentration. Glucose is vital for life and interacts with the digestive and endocrine systems. Due to this, it is imperative to maintain glucose level within the normal range under physiological condition which would otherwise leads to hyperglycemia or hypoglycemia. (Mallick and Ahsan, 2017)

For identifying these conditions, plasma glucose measurement is used. Commonly, it is widely measured for the diagnosis and management of diabetes mellitus. (Mallick and Ahsan, 2017)

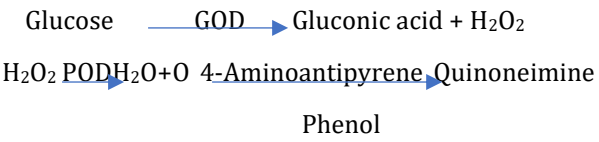
Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Good control of blood glucose levels in diabetics helps to prevent or delay the development of complications which may lead to premature disability or death from blindness, kidney failure, coronary thrombosis, stroke, bacterial and fungal infections. (Buzanovskii, 2015)

A. Methods of glucose measurement

Enzyme assays commonly used for this in vitro diagnosis are Glucose oxidase method (GOD/POD method), Hexokinase method and Glucose dehydrogenase method (Dohnal et al., 2010)

Based on the reagent stability and accuracy, glucose oxidase method is considered as the most commonly used method for plasma glucose measurement.(Mallick and Ahsan, 2017)

B.Principle of the Glucose Oxidase Method



In this reaction, glucose present in plasma is oxidized by glucose oxidase to gluconic acid with liberation of hydrogen peroxide. Hydrogen peroxide is converted to water and molecular oxygen by peroxidase. In the presence of 4- aminoantipyrene together with phenol and molecular oxygen a pink color quinoneimine is formed which is detected by measuring absorbance at 505 nm at 37°C.(Duxbury, 2004)

Most of the laboratories use commercial glucose reagent kits with traceability to international reference material for plasma glucose measurement. They are quite expensive. However, preparation of in-house glucose reagent with row materials is very convenient and cost- effective. (Zafar and Syed, 1992) When a large number of samples need to be performed, properly validated in-house reagents can be prepared and used in clinical laboratories for analytical and diagnostic purposes. The composition of in-house reagents may slightly vary from laboratory to laboratory depending on the circumstances. But, results they produced should fall within a clinically acceptable range.

The recommended formula as per the Standard Operating Procedure (SOP) in manual published by Medical Research Institute (MRI), Colombo for preparation of in – house glucose reagent (100 mL) is as follows. This is actually defined for manual method of glucose estimation.

$Na_2HPO_4.2H_2O$	1.295 g
KH_2PO_4	0. 495 g
NaN_3	0. 05 g
4-Aminophenazone	16 mg
Glucose oxidase	1800 units
Peroxidase	100 units
Phenol	105 mg
Tween 20	50 µL

Formula designed to make glucose reagent to be used on the automated analyser is given below.

Na_2HPO_4	1.25 g
KH_2PO_4	0.53 g
Glucose oxidase	2 mg
Peroxidase	2 mg
NaN_3	0.1g
4-Aminoantipyrene	15 mg
Phenol	0.11 g
Distilled water	100 mL

In this reagent, Na_2HPO_4 and KH_2PO_4 are used as buffering agents. NaN_3 is used for chemical preservation.

There are significant differences in reagent composition between these two formulas. MRI reagent is stable for about 1 month at 2-8°C whereas there stability data for the modified procedure is not checked.

The same analytical settings used on the fully automated biochemistry analyzer for commercial glucose reagents are used for in-house glucose reagent.

In this study, the above in- house glucose reagent will be compared with the commercial glucose reagent (mention the method) as the reference method or the gold standard method.

II. METHODOLOGY

A. Study design and settings

This was an evaluation study performed at Chemical Pathology Laboratory, Teaching Hospital, Karapitiya, Sri Lanka and at students' laboratory of Medical Laboratory Science department, Faculty of Allied Health Sciences, Galle, Sri Lanka. Ethical approval was obtained from the Ethics Review Committee of the Faculty of Allied Health Sciences, University of Ruhuna, Galle.

B. Study population and sample size

200 retained samples which were received to Chemical Pathology Laboratory, Teaching Hospital, Karapitiya for fasting plasma glucose measurement were used for the study, the samples were analysed within 6 hours of collection.

C. Procedure

In- house glucose reagent (500 ml) was prepared by modifying the formula developed by MRI (Medical Research Institute) stated below.

Na_2HPO_4	6.25 g
KH_2PO_4	2.65 g

Glucose oxidase	10 mg
Peroxidase	10 mg
NaN_3	0.5 g
4-Aminoantipyrene	75 mg
Phenol	0.55g
Distilled water	500mL

The fully automated biochemistry analyzer (Mindray BS-300) in Chemical Pathology Laboratory, Teaching Hospital, Karapitiya, Sri Lanka was programmed for both in-house glucose reagent and commercial glucose reagent. It was simultaneously loaded with both reagents and calibrated for both methods with the same commercial multi-calibrator from Mindray. 200 Left over samples were analyzed using in-house glucose reagent and the commercial glucose reagent in fully automated analyzer at Chemical Pathology Laboratory.

Two levels of commercial, lyophilized, assayed IQC materials were run with each batch to assure the stability and the precision of the assays. Monthly EQA samples were run to assure the accuracy of the test results.

Standard stock solution of glucose was prepared using anhydrous glucose (1000 mg/dL). An independent dilution series was prepared with 10 standards using the standard stock solution (20, 50, 100, 200, 300, 400, 500, 600, 800, 1000 mg/dL). Glucose assay was performed with each glucose standard using in-house glucose reagent and commercial glucose reagent. Calibration curves were generated separately. Linearity of the two methods were determined.

Two pools of samples were prepared and aliquoted into 20 tubes respectively in normal and pathological ranges. Glucose levels were measured using in-house reagent. Mean, standard deviation and co-efficient of variation were calculated to get the intra assay precision within batch. To obtain the inter assay precision, IQC results were obtained in 20 consecutive days.

Sensitivity, specificity and accuracy of the in-house method were checked compared to the commercial glucose reagent as the reference method.

Stability of the in-house glucose reagent was checked by assessing the performance of the in-house reagent stored at 2-8 °C, using freshly prepared QC materials over a period of three months.

Data was analyzed using SPSS version 20.0 and Microsoft Excel 2013. Paired t-test was used to calculate the significance of the mean. P value less than 0.05 was considered significant. Pearson Correlation coefficient was determined to show the correlation between two methods. Bland Altman plot was generated to evaluate the agreement of the two methods.

III. DISCUSSION AND ANALYSIS

A. Comparison of plasma glucose measurements

Total of 200 blood samples were analyzed by both in-house and commercial glucose reagents. The statistical parameters calculated for both the methods are summarized in the Table1.

Table 1. General characteristics of the two methods of glucose values of patients at Teaching Hospital, Karapitiya, Sri Lanka

Parameters	In-house Method (mg/dL)	Commercial Method (mg/dL)
Minimum	43	44
Maximum	478	454
Mean	142.28	141.44
Standard deviation (SD)	62.066	59.383
Coefficient variance (CV)	4.389	4.199

The measurements of in-house reagent showed slightly higher glucose levels as compared to the commercial reagent.

Based on the glucose concentrations obtained by both methods, the results were divided into 3 groups as follows in order to find whether there were any statistically significance in between them.

Group 1: Plasma glucose concentration below 60 mg/dL

Group 2: Plasma glucose concentration between 60 to 300 mg/dL

Group 3: Plasma glucose concentration above 300 mg/dL

The mean glucose concentrations and significance values determined by both methods are summarized in Table 2.

Table 2. Table comparing the mean glucose concentrations by both methods

	Mean plasma glucose concentration		**Significance (P value)**
	In-house method (mg/dL)	**Commercial method (mg/dL)**	
All the patients (n=200)	142.28	141.44	0.001
Group 1 (n=3)	51.5	55.0	0.395
Group 2 (n=192)	136.88	136.34	0.05
Group 3 (n=5)	386.8	373.0	0.007

The measurements of in-house reagent showed slightly higher glucose levels as compared to the commercial reagent. It was statistically significant.

Although the in-house reagent showed a lower glucose concentration in patients with glucose levels below 60 mg/dL and slightly higher concentration was observed in patients with glucose concentration 60-300 mg/dL, these differences were not statistically significant. The in-house reagent showed a higher glucose concentration compared to the commercial reagent in patients with glucose levels above 300 mg/dL, which was statistically significant. This contributed to the overall statistical significance in above table 1. This could be due to the small sample size in these groups 1 and 3 (n=3 and n=5).

Pearson correlation coefficient analysis showed very good, positive correlation between the two methods (Figure 1).

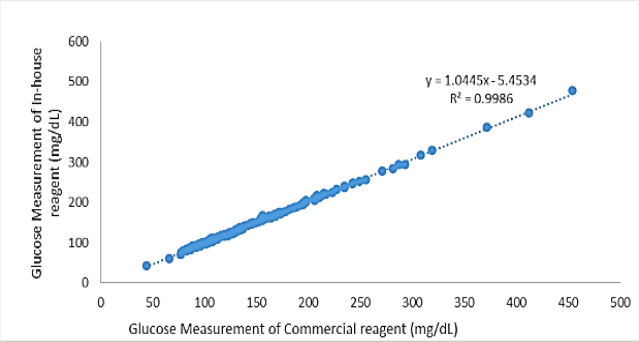


Figure 1: Linear regression graph of in-house reagent measurement vs. commercial reagent measurement

The two methods showed a strong correlation of 0.9993 according to the Pearson correlation.

Bland Altman statistical technique compares the agreement of the two methods by calculating the mean and 95% range of the differences (upper and lower limit of agreements) between the data points of the methods.

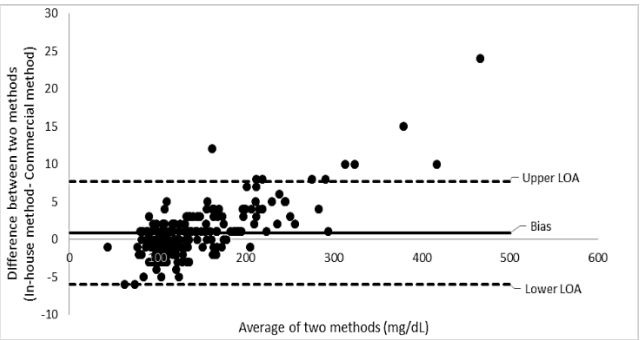


Figure 2: Bland-Altman plot

The black solid horizontal line corresponded to the bias of the two methods while the dashed horizontal lines corresponded to the 95% confidence limits of agreements (LOA). The mean measurement of in-house reagent was 142.28 mg/dL, while the mean measurement of commercial reagent was 141.44 mg/dL. The mean difference between measurements (bias) was 0.835 mg/dL. (95% CI 0.347- 1.323 mg/dL). There was thus a clear tendency for the glucose measurement of the patients to over-report their glucose measurements, by an average of 0.835 mg/dL.

The dashed horizontal lines showed 95% limits of agreement, given by the mean differences plus or minus twice the standard deviation of the differences. Approximately 95% of differences lied within this range, we could determine that the differences are normally distributed. 95% limits

were from -6.03 to 7.70 mg/dL. The differences were positively skewed after 200 mg/dl. There was a good agreement between 0-300 mg/dL.

After 300 mg/dL, the differences between two methods were dispersed beyond the upper limit of the agreement. A tendency towards increasing scatter was observed which could be due to low number of sample size in this range.(n=5)

B. Analysis of Performance characteristics

1) Determination of linearity: The reaction was linear up to 1000 mg/dL in both methods. Concentrations above the 500 mg/dL are calculated after diluting by saline automatically by the automated analyzer.

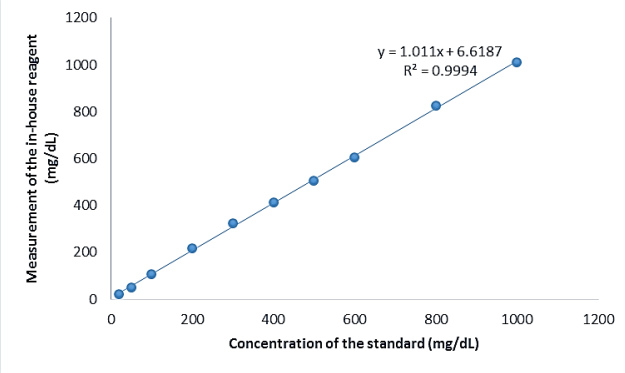


Figure 3: Calibration curve of in-house reagent

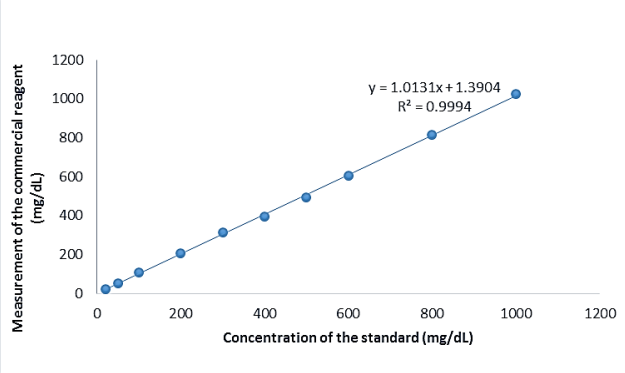


Figure 4: Calibration curve of commercial reagent

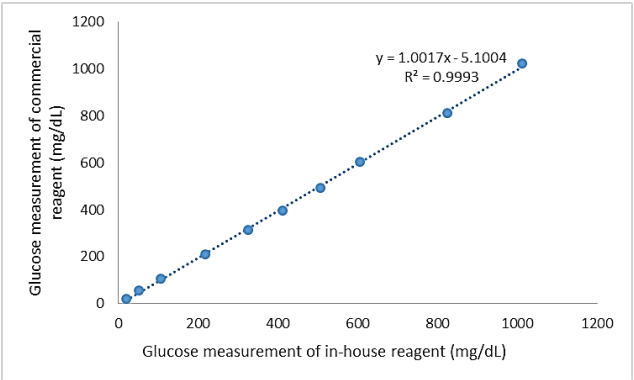


Figure 5: Correlation of the two methods using the dilution series of the standard

2) Analytical range: Analytical range is from 60 mg/dL to 300 mg/dL for in-house glucose reagent.

3) Intra- assay and Inter- assay Precision: Two pools of samples were prepared in normal and pathological levels and measured in the same day per 20 times for Intra assay precision. Quality control samples were measured in 20 consecutive days for Inter assay precision. According to that data below parameters were calculated.

Table 3.Intra -assay and Inter -assayprecision data for in-house reagent

	Intra Assay (n=20)		**Inter Assay (n=20)**	
	Normal Level	**High level**	**Normal level**	**High level**
Mean (mg/dL)	92.95	261.1	87.35	276.75
SD (mg/dL)	6.39	6.21	1.93	9.24
CV %	6.88	2.38	2.21	3.34

*4) Sensitivity, specificity and Accuracy:*According to the normal fasting plasma glucose range (60-110 mg/dL), values from both methods were categorized into 2 groups as positives and negatives.Values that are more than 110mg/dL and below 60 mg/dL were included into the positive group.Values that are within the normal range included into the negative group.Reference method was the commercial method. If from both methods values were positive, they became the real positive values and if both methods values were negative , they became the real negative.

Sensitivity, specificity and accuracy were 96.15%, 97.14% and 96.5% respectively.

Table 4.Other Characteristics in the population

	Reference Method		**Total**
	Real positive	**Real negative**	
Test positive	125	5	130
Test negative	2	68	70
Total	127	73	200

5) Stability: Stability of the reagent was checked by assessing the QC materials using the in- house reagent for a period of three months . In that three months period, reagent was stable with good performance at 2-8˚C.

C. Discussion

Glucose oxidase /peroxidase is the most commonly used method for estimation of plasma glucose in practice, due to it's a stability, reliability, (Fischl et al, 1975). It requires only small volumes of plasma and minimum reagent consumption (Sonowane et al., 1976).

Not many studies have been performed to compare the glucose measurements on in-house and commercial reagents for GOD/ POD method.

Mean values of the in-house glucose reagent and the commercial glucose reagent were 142.28 and 141.44 mg/dL; respectively for the 0- 500 mg/dL range. The measurements of in-house reagent showed slightly higher glucose levels as compared to the commercial reagent. Also, this increase was statistically significant. Other studies also suggest that slightly higher values for glucose measurements are reported for in-house reagent measurements. (Zafar and Syed, 1992). However, the mean values for 60-300 mg/dL range were 136.88 and 136.34 mg/dL. The differences were not statistically significant,

The agreement between two methods was compared using Bland-Altman plot and it showed a positive bias of 0.835 in the range from 0-500 mg/dL. It showed comparable results when compared with the commercial reagents to the manufacturers' assigned mean and range.Accuracy, sensitivity and specificity of the in-house reagent were 96.5%, 96.15% and 97.14%; respectively. Other studies have also shown similar results. (Zafar and Syed, 1992)

This in-house reagent showed a linearity up to 1000 mg/dL with the standard glucose values. However, other studies showed lower detection ranges for glucose standards with concentration up to 300 mg/dl (Passey et al., 1977) and 400 mg/dL (Sonowane et al., 1976). In this tudy, CV values for intra assay precision were 6.88% for normal level and 2.38% for pathological level. CV values for inter assay precision were 2.21% and 3.34%; respectively for normal and pathological levels.

Therefore, the prepared in-house reagent has proven better performances than previously prepared in-house reagents.

The two methods showed a strong correlation of 0.9993 according to the Pearson correlation. Taylor and Pannock, 1982 showed positive good correlation in between two methods (r=0.990).

Many studies have shown that home -made reagents are on the average 100 to 500% cheaper (Zafar and Syed, 1992). A Commercial kit (250 ml) costs Rs.1125.00 but for in-house reagent (250 ml) it costs Rs.650.00. Therefore, in-house reagents are more cost-effective than commercial reagents.

Zafar and Syed, 1992 stated glucose in-house reagent was stable for 6 weeks. This in-house reagent, has better stability over proper storage conditions over three months.

Although, the linearity has showed upto 1000 mg/dL, analytical range was from 60 mg/dL to 300 mg/dL for this method. One limitation of the study was that 192 samples were in 60-300 mg/dL range and only 8 samples were below 60mg/dL(n=3) and above 300 mg/dL(n=5). Therefore, only the results in 60-300 mg/dL range can be interpreted statistically.

IV. CONCLUSION

Although the in-house reagent showed a good linearity upto 1000 mg/dL, analytical range for this in-house reagent can be confirmed to be 60mg/dL to 300 mg/dL from this study.

Therefore, this in-house reagent can be used to measure plasma glucose level in clinical chemistry laboratory from 60 mg/dL to 300 mg/dL. Accuracy, sensitivity and specificity of the in-house reagent are

96.5%, 96.15% and 97.14%; respectively. This in-house reagent is stable for a period of three months.

Therefore, performance of in-house glucose reagent is well correlated with that of commercial reagent for the range from 60 mg/dL to 300mg/dL with good precision, accuracy and stability. The cost-effectivenes and feasible preparation procedures makes it a good candidate to assess plasma glucose levels at hospital setups where a large quantity of analyts are evaluated daily.

In this study samples with very high (>300 mg/dL, n=5) and very low (<60 mg/dL, n=3) plasma glucose values were limitted. Therefore, to validate the method for above ranges more data need to be collected. Therefore, it is recommended to expand the study for high and low values of plasma glucose levels with increased sample size in further studies to validate the method for very high and very low ranges of plasma glucose.

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