

Evaluation of Glucose Oxidase and Hexokinase Methods

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Abstract

Objective: Aim of the present study is to compare the two automated enzymatic methods to understand and familiarize the principles of method selection and evaluation

Materials and Methods: 762 individuals plasma glucose estimation were included in study.

Results: Present study the glucose concentration in plasma has been measured using glucose oxidase – peroxidase and hexokinase methods and method evaluation was performed.

Conclusion: This study has enabled to understand the concept underlying a method comparison which will help in method selection and interpretation of results obtained in the laboratory.

Keywords: Plasma Glucose, glucose oxidase – peroxidase, hexokinase, NCCLS - National Committee for Clinical Laboratory Standards, ISO - International Organization for Standardization

INTRODUCTION

Glucose is the major carbohydrate found in the blood and a chief source of energy in human body. The nervous system, including the brain, totally depends on glucose from the surrounding extra cellular fluid (ECF) for energy (1). Many analytical procedures are used to measure blood glucose concentration. In the past, analyses were often performed with relatively non specific methods that resulted in falsely

increased values. Almost all commonly used techniques are now enzymatic and older methods, such as photometric or oxidation-reduction techniques, are rarely used (2).

The introduction of new or revised method is a common occurrence in the clinical laboratory. The evaluation of new methods is guided by several schemes that outline experimental procedures and statistical techniques (3). NCCLS and ISO have developed several documents related to method evaluation (1). The technical information of new test includes analytic sensitivity, specificity (4) which can be understood by analysis of an analyte by two different methods. Medical requirements for performance can best and most easily described in terms of the total analytical error (5). Total analytical error is compared with allowable error (6). In addition to EA, it is necessary to specify medical decision level (Xc).

In this study we describe the quantitative evaluation of plasma glucose by glucose oxidase-peroxidase method and hexokinase method. (i.e.) we compare the two automated enzymatic methods to understand and familiarize the principles of method selection and evaluation (7).

Objective

- To understand the ability and to judge the acceptability of an analytical method in a clinical laboratory
- To monitor an assay total analytical error using quality control sample.

MATERIAL AND METHODS

Study group consisted of 762 individuals who had requested for plasma glucose estimation at central hospital laboratory services, Sri Ramachandra medical college and hospital, Chennai. Whole blood was collected in sodium fluoride vacutainers because it exerts its preservative action by inhibiting the enzyme systems involved in glycolysis. Plasma was got after centrifugation was used for analysis of glucose by glucose oxidase-peroxidase and hexokinase method.

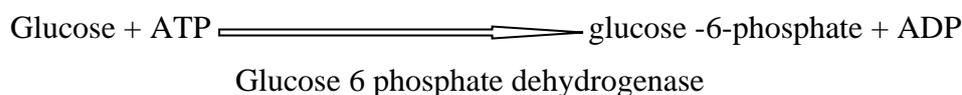
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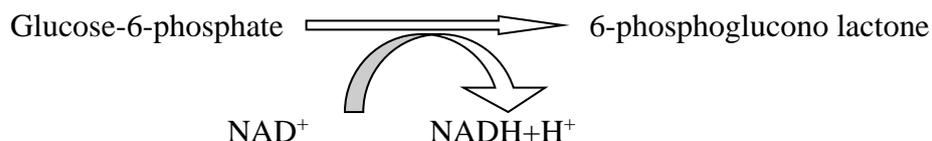
Principle of glucose oxidase-peroxidase (GOD-POD) method



The colour is measured at 505nm

Principle of Hexokinase method





The colour is measured at 340 & 383nm

Instruments – Dimension RXL Max and Kone Lab 60i

Correction between two methods was analysed for the study group

Total analytical error was calculated for normal control samples (NQC) using the formula.

$$TE = SE + RE$$

Systemic error was also calculated using the formula.

$$|(y_0 + m X_0) - X_0|$$

m = slope

y₀= intercept

X₀=decision level.

Random error (RE) is calculate using formula

$$2.58 \times S$$

2.58 – multiplier to calculate random error.

S – Standard deviation.

EA for glucose is obtained from CCLA 88 (8) for glucose- target value+10%. Recovery a measures of accuracy is determined by adding a small aliquots of known amount to a sample. (Calibrators of concentration 100 and 200mg% glucose solution were used). The linearity range was checked using calibrators (0-600mg %) for the both methods

Statistical analysis

The results were expressed as mean \pm standard deviation. The co-efficient of variation was calculated for the study groups and control samples. The correlation between both the methods was calculated using Pearson correlation coefficient. The systemic error for normal quality control samples was calculated using Deming regression.

RESULTS AND DISCUSSION

The study group consists 762 individuals who had requested for plasma glucose estimation at the central hospital laboratory services of Sri Ramachandra medical college and hospital, Sri Ramachandra University, Chennai. The entire group was further divided in to five groups based on their plasma glucose concentration.

Table No: 1 Group based on the plasma glucose concentration.

Groups	Plasma glucose concentration	Number of samples
Entire Group	44-582 mg%	n= 762
Group I	<100 mg%	n = 165
Group II	101–200 mg%	n = 509
Group III	201-300 mg%	n = 83
Group IV	301- 400 mg%	n= 21
Group V	401-500 mg%	n= 4

The methods for glucose measurements based on the reducing properties of glucose, because of interference by so called saccharoids (non reducing substances), systematically overestimates the true plasma glucose concentrations as compared with enzymatic procedure(9). In the present study the glucose concentration in plasma has been measured using glucose oxidase – peroxidase and hexokinase methods.

Table No: 2 Distribution of samples based on glucose concentration

Plasma glucose concentration	Plasma glucose (mg %)		Correlation (r)
	GOD POD	HK	
Entire Group 44-582mg%	142±61.99	144±61.93	0.99
Group I <100 mg%	91.12 ± 22.62	92.03±22.90	0.95
Group II 101–200 mg%	135.32±26.00	136.1±26.32	0.96
Group III 201-300 mg%	271.84 ± 25.6	272.87± 25.87	0.93
Group IV 301- 400 mg%	334.71±24.01	335.62± 25.01	0.93
Group V 401-500 mg%	437.75 ± 35.1	437.8 ± 35.3	0.99

GOD-POD – Glucose oxidase peroxidase, HK- Hexokinase

The results of our study indicate that a minimum bias was obtained by both the methods over various range of plasma glucose concentration. The good correlation between hexokinase and glucose concentration (<100mg% to 500mg%) were found to be in accordance with the studies reported by Meena sonowane et al (10) who reported a correlation value $r = 1.00$ between their two methods. Both the enzymatic methods have advantages and disadvantages. The stability of glucose oxidase reagent is excellent and refrigeration during the course of analyzer is unnecessary (10). The cost per test for glucose oxidase is low when compared to hexokinase method. Jose A. rodrigue – caqstelion et al, has reported a correlation value $r = 0.98$ between both the method up to 300mg%. Small interferences from lipemic sera and from high concentrations of ascorbic acid with glucose oxidase method have been reported by them (11).

Table No: 3 Quality Control (QC) sample

Quality controls	GOD-POD plasma glucose (mg %)		HK plasma glucose (mg %)	
	MEAN \pm SD	CV (%)	MEAN \pm SD	CV (%)
Normal N=19	94.42 \pm 4.31	4.56	97.41 \pm 4.3	4.42
Abnormal N=19	295.47 \pm 17.72	5.01	297.52 \pm 17.84	5.12

In this study, coefficients of variation for plasma glucose in the normal and abnormal quality control samples were similar. Koch TR and Nipper has supported that the between - day co-efficient of variation on auto analyzer I and auto analyzer II on Trinder's method were 1.6% (mean glucose 198%) and 2.6% (mean 228%) respectively. They have also shown the correlation $r = 0.974$, when Trinder's method was compared with manual blanked hexokinase in auto analyser I and value $r = 0.991$ when analysed in auto analyser II. The day to day co-efficient of variation of glucose by glucose dehydrogenase method on kinetic mode was 2.54% (mean glucose 99.9mg %) (12). The estimates of random and systemic error are combined to provide estimates of the total analytical error. In our study, the total error for normal quality control sample has been represented in Table No 4.

Table No: 4 Total Error for quality control sample

Normal QC	Glucose oxidase			Hexokinase		
	SE	RE	TE	SE	RE	TE
	-3.65	11.11	7.46	-3.65	11.09	7.44

In assessing the acceptability of a clinical method, its analytical performance should be judged relative to the clinical requirements for the test results. For glucose, several definition of clinical requirement (performance standard PS) may be stated, based on the suggestions of medical usefulness requirements by Barnett and on error specification from the FDA(1). Four performance standard can be defined as an allowable error limit of 100mg/L at critical glucose concentration of 500mg/L (PS I) and 1200mg/L (PS II). An allowable error of 1200mg/L in the concentration range of 1500-3000mg/L (PS III) and an allowable error of 400mg/L for glucose concentrations excluding 3000mg/L (PS IV).

In our study the total analytical errors obtained by both the methods were similar as indicated in the Table No 4. This is in accordance with the results reported by Passey et al (5) and Carl C. Garber et al (13). The analytical recovery of glucose by both the methods were evaluated by adding known volumes of 100mg% and 200mg% glucose

aqueous standards to normal QC. The results of this recovery experiment have been represented in the Tables No 5. A minimum of recovery percentage (88%) was obtained in our study.

Table No: 5 Recovery

NQC (Hexokinase) Dimension RXL Max	Glucose measured (mg/dl)	Glucose added (mg/dl)	Recovered Glucose (mg/dl)	Recovery %
Sample I	73	-	-	-
Sample II	90	17	17	100
Sample III	104	33	31	94
N QC (GOD) Kone lab 60	Glucose measured (mg/dl)	Glucose added(mg/dl)	Recovered glucose(mg/dl)	Recovery %
Sample I	77	-	-	-
Sample II	92	17	15	88
Sample III	111	33	34	103

Carl C. Garber (13) has reported 99.7% for co-immobilized hexokinase method and 100.2% for the glucose oxidase method. The linear range also known as analytic or dynamic range is the concentration range over which the measurement concentration is equal to the actual concentration without modification of the method. The wider the linear range, the less frequent will be specimen dilution. In the present study the linearity check was performed by both the enzymatic methods using aqueous glucose calibrators ranging from (0 to 600mg %). The results are shown in Fig No 1 and Fig No 2.

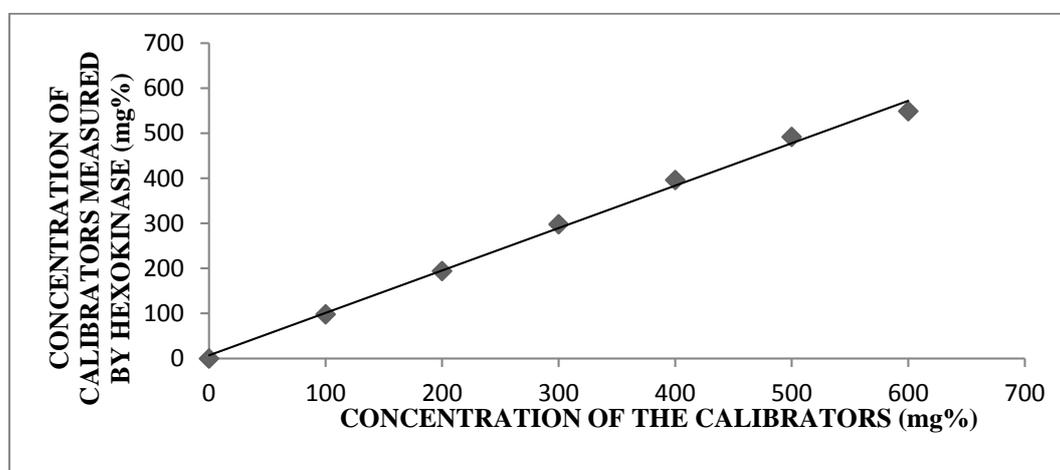


Fig. No 1: Linearity check for calibrators by Hexokinase method

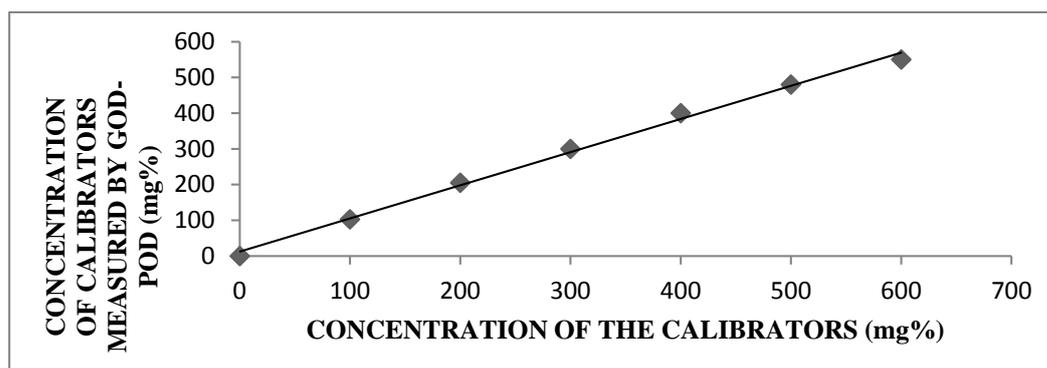


Fig. No2: Linearity check for calibrators by glucose oxidase-peroxidase method

The studies by Stanley J. Miskiewicz et al (14) has indicated that glucose oxidase-peroxidase – ABTS system provides an accurate, precise, sensitive method with an excellent linearity upto 500mg/100ml. The linearity of Trinder's method by Koch (15) was reported to be up to 500mg% and a recovery of 98%. There were no significant deviations of results within the range of concentration encountered physiologically. The study by Rendolf A. Lutz and Jurg fluckiger (8) has shown that the kinetic determination of glucose by glucose dehydrogenase reaction in comparison with glucose oxidase and hexokinase method was linear upto 300mg%. This study has enabled to understand the concept underlying a method comparison which will help in method selection and interpretation of results obtained in the laboratory.

CONCLUSION

The concept of evaluating a method that is presented here is not new, the mean value of a group represents accuracy of a method. The standard deviation of a group represents precision of a method. Routinely used methods in the clinical labs are glucose oxidase and hexokinase which is also evident from good correlation obtained in this study. The total error obtained reveals that both methods are acceptable for measuring glucose. The linearity and recovery are in accordance with manufacturer claims. This study has enabled to understand the concept underlying a method comparison which will help in method selection and interpretation of results obtained in the laboratory.

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