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Original Research Article

A prospective comparative study of glucose estimation by hexokinase and glucose oxidase-peroxidase methods

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ABSTRACT

Aims and Objectives: Error analysis of the comparative study of glucose estimation with the comparison and regression analysis.
Introduction: Glucose is the major carbohydrate found in the blood and a chief source of energy in human body. Estimation of glucose is very important in clinical diagnosis of hyperglycemia, hypoglycemia and normoglycemia. Accuracy of test results are very important, requires considerable effort and cost. For introducing in the laboratory the test method has to be evaluated by the process called quality control. Method selection and evaluation is important in improving efficiency.
Materials and Methods: The preferred sample for Glucose assay is fasting serum or plasma. A total of 105 samples are analysed for the quantitative estimation of blood glucose by the reference method.
Discussion and Results: In the present study comparison between a test method (Glucose oxidase-peroxidase) and a reference method (Hexokinase), the errors are less than TEa. Mean value of Hexokinase method is 311.36 ± 137.315 and mean value Glucose oxidase-peroxidase method is 291.87 ± 133.412. p-value on comparison = 0.00014. With this the test method can be an alternative to reference method in terms of accuracy and precision and analytical sensitivity and specificity.
Conclusion: Glucose oxidase-peroxidase method is also a suitable method for the quantitative estimation of glucose in need of clinical requirements.

1. Aims and objectives

To study the correlation and regression analysis between reference method (Hexokinase) and test method (Glucoseoxidase-peroxidase) in hyperglycemic and normoglycemic patients attending the central lab, Dept. of Biochemistry - GGH, Guntur.

2. 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] Definition of diabetes requires measurement of glucose in samples of whole blood, serum or plasma.[2] 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.[3] Estimation of glucose is very important in clinical diagnosis of hyperglycemia, hypoglycemia and normoglycemia. Accuracy of test results are very important, requires considerable effort and cost. For introducing in the laboratory the test method has to be evaluated by the process called quality control.[4] The value of clinical laboratory service is to provide reliable, accurate test results. At the heart of providing these services is the performance of a testing method.[5] Method selection and evaluation is important in improving efficiency. Analytical sensitivity, analytical specificity interfering substances and estimation of impression and inaccuracy are possible with method comparison.[6] The preferred specimen for glucose assay is fasting serum or plasma. In the fasting state the difference between arterial, capillary and venous blood- glucose levels

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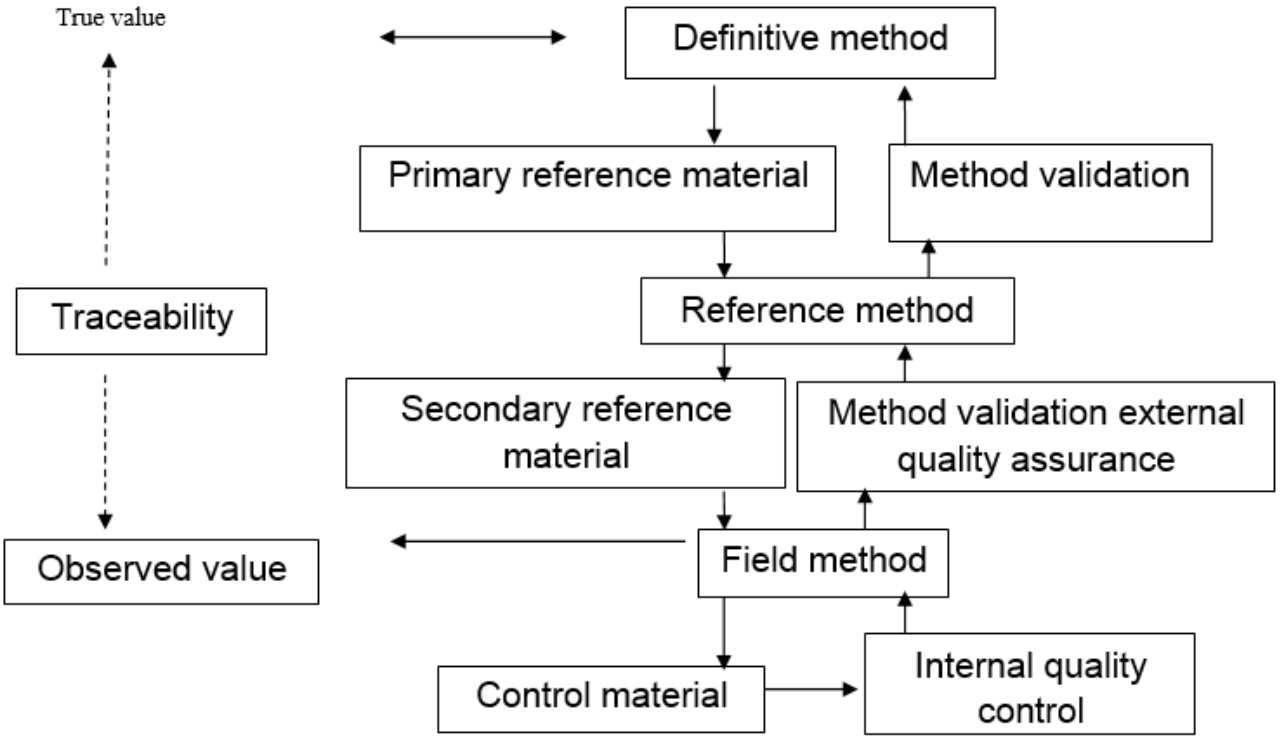

Diagram 1: Accuracy based measurement system showing relationship between referencemethods and materials

are small (only 2-3mg/dl).[7]

3. Materials and methods

The preferred sample for Glucose assay is fasting serum or plasma. A total of 105 samples are analysed for the quantitative estimation of blood glucose by the reference method.

3.1. Inclusion criteria

Diabetic patients irrespective of the cause and normo-glycemic patients.

3.2. Exclusion criteria

Severe hypoglycemic conditions and hemolysed and lipaemic samples were excluded.

3.3. *Principle of glucose oxidase-peroxidase method*

$$\beta - D\ Glucose + O2 + H2O \xrightarrow{Glucose\ Oxidase} Gluconic\ Acid + H2O2$$

$$H2O2 + Reduced\ chromogen \xrightarrow{Peroxidase} Oxidised\ chromogen + H2O$$

(0-dianisidine)

3.4. Sources of errors

Glucose oxidase is highly specific for β-D Glucose. Other reducing substances like uric acid, ascorbic acid, Glutathione, Bilirubin may inhibit the reaction, resumed through competition with chromogen for H_2O_2, resulting in a negative bias or compounds may be present that oxidize the indicator dye resulting in a positive bias[8]Glucose + ATP Glucose-6-Phosphate + ADP Glucose-6-P + NADP 6 -Phosphogluconate + NADPH + H

3.5. Principle of hexokinase method

$$Glucose + ATP \xrightarrow{Hexokinase, Mg2+} Glucose - 6 - Phosphate + ADP$$

$$Glucose - 6 - P + NADP \xrightarrow{G6PD} 6 - Phosphogluconate + NADPH + H+$$

The absorbance of NADPH + H^+ measured at 340nm directly proportional to glucose concentration. The most commonly used procedures for glucose analysis employ enzymes as reagents to increase analytical specificity.

3.6. Sources of errors

Phosphate esters and enzymes released from red blood cells may react to produce changes in the $NADP^+$ concentration. Therefore hemolysed samples (>0.5 mg/dl Hb) cannot be

used. Ascorbic acid and uric acid do not interfere. The main disadvantage in the past with Hexokinase method was the lack of reagent stability.

3.7. Evaluation of new procedure

If the method is a reference method without bias and non- specificity, the target value equals to the true value, given a field method some bias or non-specificity may be present, and the target value and true value are likely to differ somewhat.[8] Method evaluation is used to verify the acceptability of new method prior to reporting patient results.

New procedure is easier to perform, is less costly or has other advantages excluding greater accuracy and precision over older procedures.[9]

A flowchart on the process of method selection, evaluation, and monitoring.

The determinations in the method evaluation are

1. Imprecision
2. Inaccuracy

Imprecision is the dispersion of repeated measurem ents around a mean (True value) due to analytical error. Random analytical error is the cause of imprecision in a test.

3.8. Random error

Error varies from sample to sample. Causes include instrument instabilities, temperature variations, reagent variation, handling techniques and operator variables.

In precision study, 2 controls are run twice a day for 20 days. Imprecision is estimated in our study in which multiple aliquots of the same specimen (with a constant concentration) are analyzed repetitively. Random analytical error is the cause of imprecision. Precision determined by repeated analysis. Test used to determine Random error is Replication experiment.

3.9. Inaccuracy

Inaccuracy or the difference between measured value and its actual value is due the presence of the systematic error. Systematic error can be due to constant error and proportional error. Inaccuracy determined by

1. Comparison of method study
2. Recovery study
3. Interference study

3.10. Constant error

Type of systematic error in the sample direction and magnitude. Magnitude of change is constant and not dependent on the amount of analyte. In the absence of proportional error constant error is equal to y-intercept. It is also equal to the bias between the methods. The test used to determine the constant error is interference experiment.

3.11. Proportional error

The type of systematic error where the magnitude change as a percent of the analyte present. Error dependent on analyte concentration. Error between methods that increases as a assay value increases. In other words the difference between results obtained on the test and reference method gets larger as the level increases. Proportional error is often due to calibration difference between methods being compared.

In our present COM studies recommended by Westgard et al[10] and CLIA that a test method (Glucose oxidase-peroxidase) compared with a reference method (Hexokinase method). Daily analysis of five samples runs by each method for about 20 days.[11]

A plot of test method data (y-axis) versus the comparative method (x-axis) data was done.[12]

4. Statistical analysis and Results

Linear regression analysis is more useful than the t test for evaluating comparison of method (COM) studies.[13]

The linear regression technique for method comparison is typically employed when a new method or instrument is being introduced into a laboratory. Linear regression is widely employed in clinical chemistry, and as such receives theoretical coverage in a variety of educational texts.[14–16]

The linear regression defined by the equation y= mx+b. The line of best fit thought he data points is obtained by least square linear regression and can be calculated either manually or with statistical software(SPSS).

The calculated correlation coefficient(r) represents the linear correlation between the two methods. The correlation coefficient reveals the relationship of the two methods being compared.

Regression equation for method comparison

Number of samples (N) =105

y = 9.25 + 0.97x

Slope (m) = 0.97

y intercept (b)= 9.25 mg/dl

r =0.991

The systematic error (SE) at a given medical decision concentration (Xc) is then determined by calculating the corresponding Y value (Yc) from the regression line. Then taking the difference between Yc and Xc as follows.

Yc = b + mx

Systematic error = Yc – Xc

Systematic error for glucose at a given medical decision concentration of Xc= 200mg/dl.

= 0.970 x 200 + 9.25= 203.5mg/dl

- Xc = 203.5-200 = 3.5 mg/dl (systematic error)

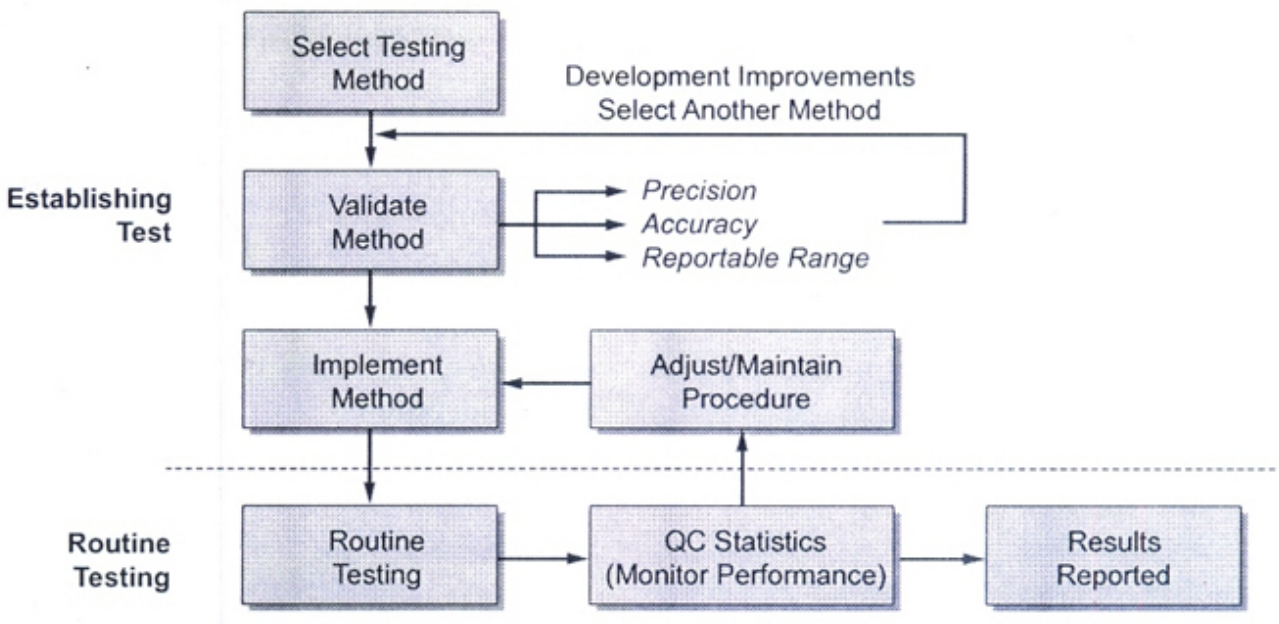

Diagram 2:

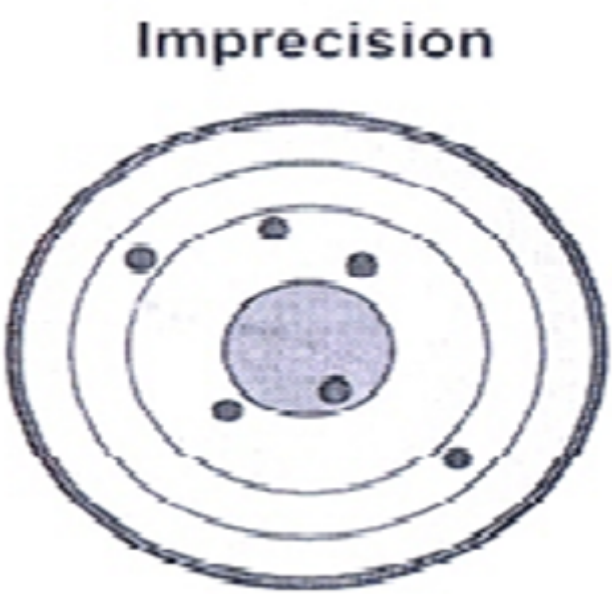

Diagram 3:

Table 1: Quality control statistical parameters

Method	**Glucose oxidase-peroxidase Method (mg/dl)**		**Hexokinase (mg/dl)**	**Method**
	Standard (100)	Standard (200)	Control 1/ base value	Control 2/ base Value
Mean	99.545	199	107.8 / 112.0	274 / 285.0
SD	0.784	2.54	2.29 / 8.15	5.68 / 22.00
CV %	0.783	1.276	2.12 / 7.28	2.07 / 7.72

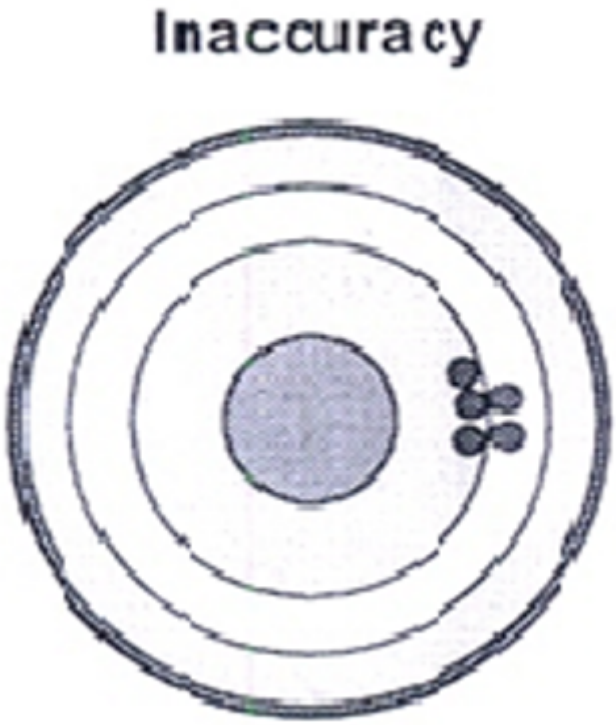

Diagram 4:

Table 2: Descriptive statistics

Descriptive Statistics			
	Mean	**Std. Deviation**	
AU480 Hexokinasemethod	311.36	137.315	105
ERBA Glucoseoxidase peroxidase method	291.87	133.412	105

Table 3: Correlations

Methods		**AU480 Hexokinase method**	**ERBA Glucose oxidase-peroxidase method**
AU480 Hexokinase method	Pearson Correlation	1	.995**
		105	105
ERBA Glucose oxidase-peroxidase method	Pearson Correlation	.995**	1
		105	105

Table 4: Statistical parameters in method comparison

M	**(mg/dl)**	**Sy/x (mg/dl)**	**Bias (mg/dl)**	**F**	**R**
0.97	9.25	3.11	-19.49	1.059	0.991

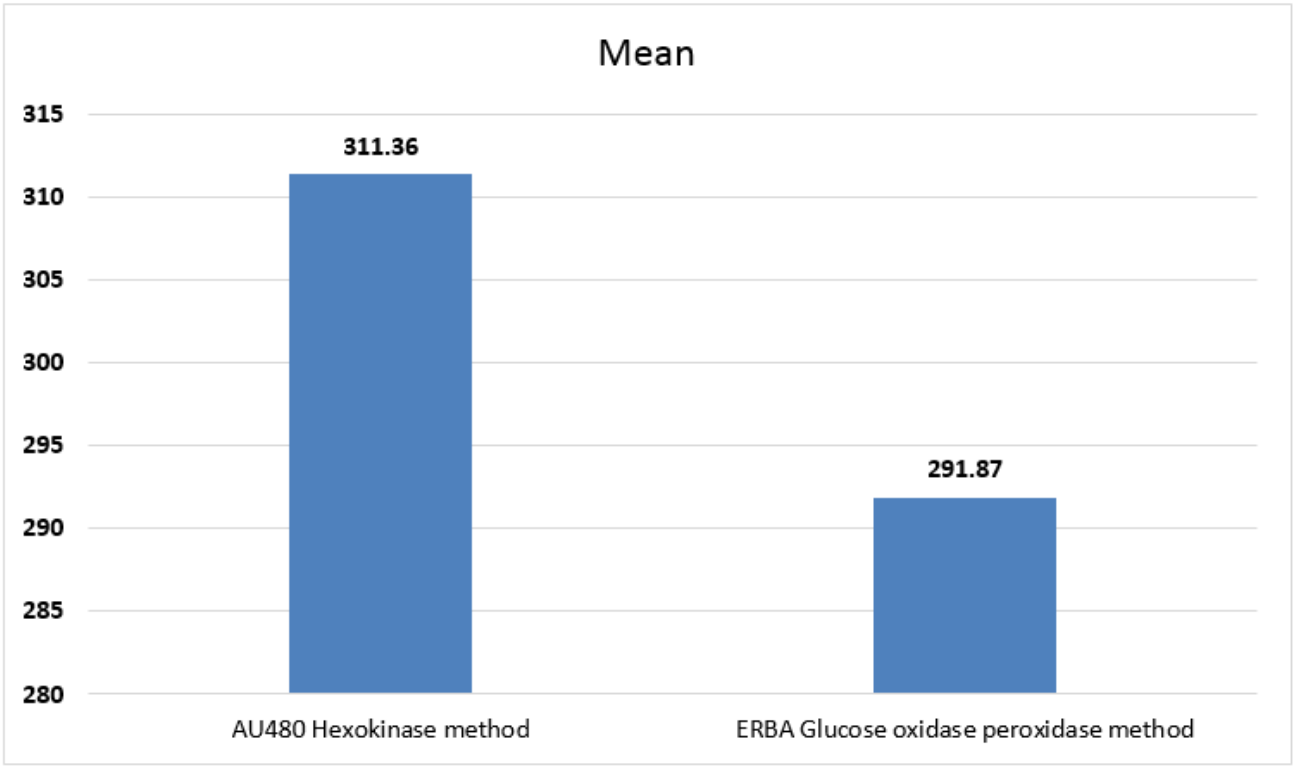

Fig. 1:

4.1. Evaluating correlation coefficient

Squaring r r^2 = 0.991 x 0.991

Multiplied remainder by 100

Subtracting the result from 1.000

=1.000-0.991x0.991x100

= 1.7%

Method GOD-POD has r closer to 1.000 and 1.7% results are out of control compared to reference method.

Bias = Test method mean-reference method mean gives rise to average of bias.

291.87-311.36= -19.49

This shows negative bias for the test method (Glucose oxidase-peroxidase). Negative bias indicates that the test values tend to be lower than the reference value. Bias is a type of constant systematic error.

Proportional error= 1.000-0.97= 0.03 x 100 = 3%

Standard error of estimate (Sy/x) is a statistical parameter and is calculated according to equation that indicates the "scatter" of data points about the calculated regression line. A large Sy /x value suggests there is a appreciable random error between methods being compared. In the present study, Sy/x =3.11mg/dl.

$Sy/x = \sqrt{\sum \frac{(yi-yi)2}{n-2}}$

- Xc $\pm$ 2(Sy/x) or

3.5 $\pm$ 2 (3.11) = 9.72(-2.72 to +9.72)

Total error= 9.72 mg/dl.

4.2. Recovery studies

Recovery studies meant for measurement of accuracy. In this study a small aliquot of concentrated analyte is added into a patient sample and then measured by Glucose oxidase-peroxidase method being evaluated. The amount recovered is the difference between the sample measured with added concentration and the baseline measured patient sample.

$Recovery\% \ \frac{Sample\ measured\ with\ added\ concentration - baseline\ measured}{Concentration\ added} \times 100$

In this recovery study samples are having recoveries in acceptable percentage (90% to 110%).

5. Discussion

In the present study the accuracy of test method determined by bias which is a negative bias (-19.49 mg/dl). In recovery study the percentage of recovery is from 94 – 104% which is acceptable. The precision of a test method is determined by standard deviation for Glucose oxidase-peroxidase method (SD 0.784, CV 0.783 for a 100mg standard of glucose) and (SD 2.54, CV 1.276 for a 200mg standard of glucose. In Hexokinase method, control (SD is 2.29, CV 2.12) and control2 (SD is 5.68, CV 2.07). F value

Table 5: Recovery studies

Sample	Baseline value (mg/dl)	Glucose added (mg)	Glucose recovered (mg/dl)	% recovery
Sample 1	74	100	173	99%
Sample 2	63	100	157	94%
Sample 3	136	100	240	104%

of the comparative study is 1.059. In accordance with the studies reported by Meena Sonowane et al.[17] who reported correlation value r=1.00 between the two methods. Jose A. Rodriguez-Castellon et al[18] has reported a correlation value r=0.98, between both the methods up to 300mg%. If r^2 is less than 0.99 then alternative analysis should be used.[19] Statistical comparison demonstrates excellent agreement between Hexokinase and Glucose-oxidase methods.[20]

6. Limitations of study

Interference studies not done.

7. Conclusion

The deeper concept of method comparison is to rule out the error measured with the test method (Glucose oxidase-peroxidase method).

Total error= Random error + Systematic error

Random error= 2.58 x S(sd)

Systematic error= Yc – Xc from the equation Yc = B + Ax

As per the clinical laboratory improvement amendments of 1988 (CLIA 88)[12] glucose performance standards is greater than target ± 6mg/dl or ±10% (total allowable error).

On comparison with Hexokinase method, Glucose oxidase-peroxidase method is having a Total error which is less than the TEa.

The present study reveals that the two methods are acceptable for measuring glucose.

8. Source of funding

None.

9. Conflict of interest

None.

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