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A comparative analysis of Point of care lateral flow immune assay (LFA) with routine and optimized laboratory assays for the detection of HbA1c levels in a human whole blood specimen

Nagababu Pyadala*[1,2], Prudhvi Chand Mallepaddi[3], Soumendra Nath Maity[1,4], Ravi Kumar B N[2], Vijayaraghavan R[5], Rathnagiri Polavarapu[6]

[1]Department of Research, Saveetha University, Thandalam, Chennai, Tamil Nadu, India
[2]Department of Biochemistry, MNR Medical College and Hospital, Sangareddy, Telangana State, India
[3]Department of Clinical Research Laboratory, Genomix Molecular Diagnostics Pvt. Ltd, Kukatpally, Hyderabad, Telangana, India
[4]Department of Microbiology, Malla Reddy Institute of Medical Sciences, Suraram, Hyderabad, Telangana, India
[5]Department of Research, Saveetha University, Thandalam, Chennai, Tamil Nadu, India
[6]MNR Foundation for Research and Innovation, Sangareddy, Telengana, India

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ABSTRACT

The present study was aimed to compare the HbA1c point of care LFA test kit with routine and clinically proven, optimized methods for detection of HbA1c in whole blood samples. A total of 90 samples from patients with DM tested with newly developed LFA test kit and other analytical techniques. The results were analyzed for comparison and correlation of the HbA1c values to classify DM patients according to their glycemic control. Through regression analysis, the correlations between the results of HbA1c LFA and Alere HbA1c kit was found to be R2= 0.977 and for the HbA1c LFA and Biohermes A1CEZ kit was found to be R2= 0.980. The correlation between HbA1c LFA and ERBA Glycohemoglobin kit was found to be R2= 0.965 and for the HbA1c LFA and BIO-RAD variant HbA1c kit (HPLC) was found to be R2= 0.911. Based on the results obtained so far through newly developed LFA, compared with gold standard and other optimized methods. The LFA has proved to be rapid, simple, inexpensive, the point of care method with excellent sensitivity and specificity.

* Corresponding Author

Name: Nagababu Pyadala
Phone: +91-9061076459
Email: saisailesh.kumar@gmail.com

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INTRODUCTION

Globally the incidence of diabetes mellitus (DM) increasing day by day and became an epidemic which affecting one in twelve (Aguiree *et al.,* 2013). DM became one of the leading cause of death and disability. In India, the prevalence of DM rapidly increasing day by day, especially Type 2 DM (NIDDM) which reduces the quality of life through its complications. Incidence is increasing day by day due to obesity and reduced physical activity. Because of this World Health Organization (WHO), declared India as a diabetes capital of the world (Nagababu *et al.,* 2016; Sarala *et al.,* 2016). People suffering from DM should monitor their blood glucose levels regularly, due to poor management of blood glucose levels results in dysfunction and damage to various organs especially, eyes, blood vessels, nerves, heart, and kidneys. Therefore regular monitoring is recommended for treatment and diagnosis of DM (Kiran et al.,2017; Sarala *et al.,* 2017). According to Heller *et al.,* (2008) to monitor

blood glucose, we required minimum three to four test a day to determine the blood glucose, which may fluctuate the blood glucose concentrations. For this American Diabetes Association (ADA) standard of medical care suggested screening of glycosylated haemoglobin (HbA1c ≥ 6.5 %) for the management and diagnosis of DM (Heller *et al.,* 2008). Worldwide HbA1c used as a biomarker for long-term glycemic control. Which was used as a screening test for not only for management and monitoring but also used for identification of risk of developing complications (Genuth *et al.,* 2003; Bodor *et al.,* 1992; Sato *et al.,* 2009; Diabetes Care; Edelman *et al.,* 2004). In clinical practice determination, of HbA1c was widely used to monitor glycemia in diabetic patients and measurement of HbA1c has a high demand in the clinical laboratory. Several analytical methods are developed and recommended to measure the HbA1c in whole blood. Some of those techniques are borate chromatography (Frantzen et al.,1997; Psotova *et al.,* 1995), ion-exchange chromatography (Eckerbom *et al.,* 1994), electrophoresis (Jenkins et al, 2003), immunoassays (Ikeda *et al.,* 1998; Metus *et al.,* 1999) and newly developed Lateral flow immunoassay (LFA) . In clinical settings choosing of these methods depends upon the measurement of HbA1c marker; should be simple, Point of care (POC), and economical; should get results in highly precisely with excellent sensitivity and specificity. Among the different methods used to measure the HbA1c showed many analytical problems and these methods were technically demanding and time–consuming. So the present study aimed at comparative evaluation of newly developed LFA with routine and optimized laboratory assays for the detection of HbA1c levels in whole human blood.

METHODS AND MATERIALS

The majority of this study was performed at Central research lab, MNR Medical College & Hospital, Sangareddy, Telangana. A total of 90 whole blood samples collected in vacutainer with EDTA from diabetic patients attending the diabetic clinic during the period from January 2016 to December 2016. 8 ml of whole blood collected from anti- cubital vein from each diabetic patient. The samples were kept stored at 4^0C till they were analyzed. Diabetic patients selected after taking detailed clinical history and brief medical examination. The institutional ethical committee approved this study.

The 90 whole blood samples tested by in-house haemoglobin A1c lateral flow immune assay (HbA1c LFA), BIO-RAD- D-10 system for HbA1c (Ionic exchange high-pressure liquid chromatography (HPLC), Alere AS 100 HbA1c, Biohermes A1C EZ (Boric acid affinity chromatography) and ERBA glycohaemoglobin kit (Ion-exchange Resin method).

All the samples were categorised in to four distinct ranges: 4% (20 mmol/mol) to 6% (42 mmol/mol), 6% (42 mmol/mol) to 8% (64 mmol/mol), 8% (64 mmol/mol) to 10% (86 mmol/mol), and > 10% (86 mmol/mol). All the samples were analyzed with each instrument, simultaneously, and performed regression analysis.

Statistical analysis

The statistical analysis was performed using SPSS software version- 16 (SPSS Inc, Chicago).

RESULTS

A total of 90 samples analyzed for HbA1c estimation. There were 54 males and 36 females as shown in figure 1 and table 1. HbA1c measured by newly developed hemoglobin A1c LFA. Based upon the signal intensity of the test lines measured by ESE lateral flow reader HbA1c levels are estimated. We compared the results obtained by our lateral flow assay to the results obtained from BIO-RAD-D-10, Alere AS 100 HbA1c, Biohermes A1C EZ and ERBA glycohaemoglobin kit by regression analysis as shown in figure 2-5.

Table 1: Gender and Age wise distribution of diabetic patients

Age (years)	Diabetic Patients		
	Male	Female	Total
30-40	07	08	15
41-50	22	17	39
51-60	14	06	20
61-70	11	05	16
Total	54 (60%)	36(40%)	90(100)

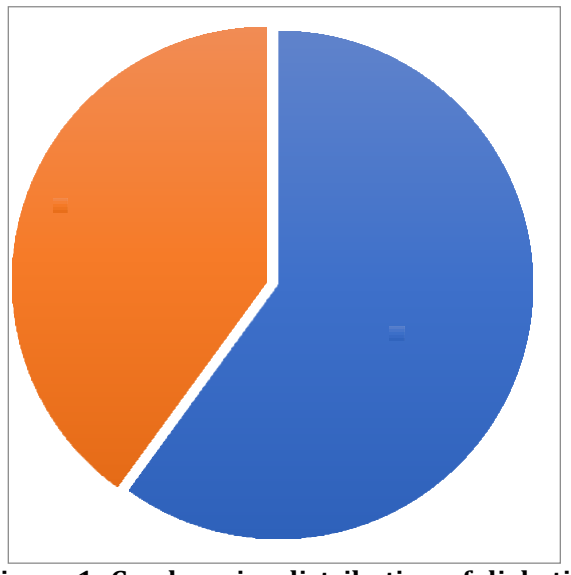

Figure 1: Gender wise distribution of diabetic patients

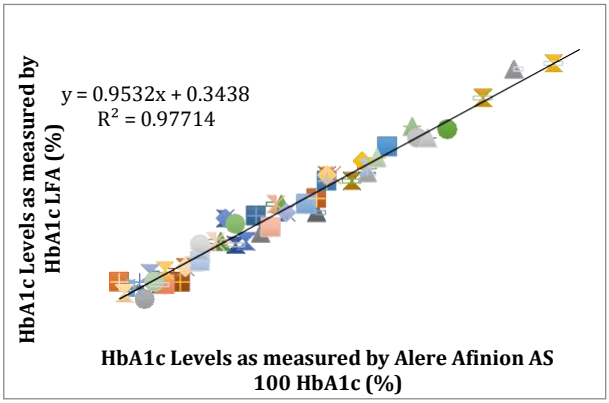

Figure 2: HbA1c LFA vs Alere Afinion AS 100 HbA1c

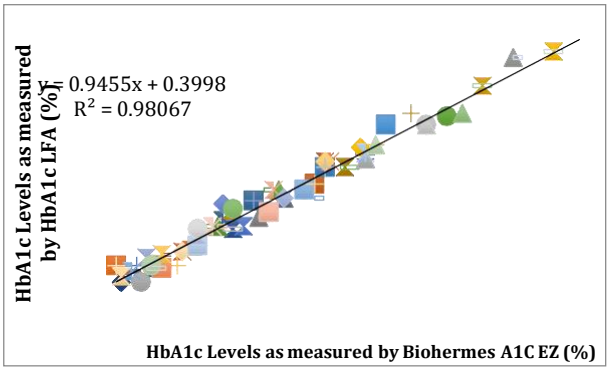

Figure 3: HbA1c LFA vs Biohermes A1C EZ

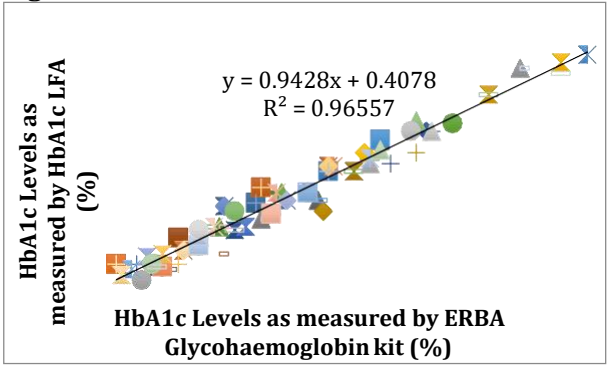

Figure 4: HbA1c LFA vs ERBA Glycohaemoglobin kit

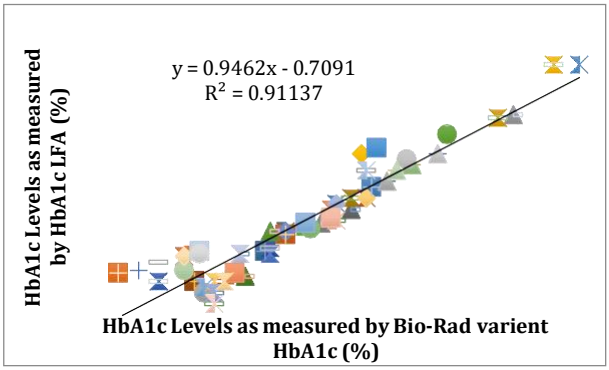

Figure 5: HbA1c LFA vs Bio-Rad varient HbA1c

DISCUSSION

All of the 90 samples tested with LFA and analyzer systems showed a good correlation with HPLC which is a standard gold method to measure HbA1c. The result obtained from newly developed LFA compared with the results obtained from other analytical methods like Alere Afinion AS 100 HbA1c, Biohermes A1CEZ, ERBA glyco haemoglobin kit and BIO-RAD variant HbA1c kit (HPLC) by regression analysis as shown in figure 2 (2a,2b,2c &2d). These analytical methods are optimized and clinically proven methods, which are commercially available. Through regression analysis, the correlations between the results of HbA1c LFA and Alere HbA1c kit was found to be R2= 0.977 (Figure 2a), and for the HbA1c LFA and Biohermes A1CEZ kit was found to be R2= 0.980 (Figure 2b). The correlation between HbA1c LFA and ERBA glycohaemoglobin kit was found to be R2= 0.965 (Figure 2c) and for the HbA1c LFA and BIO-RAD variant HbA1c kit (HPLC) was found to be R2= 0.911 (Figure 2d). The correlation between newly developed LFA and Standard methods are quite similar. According to Martina *et al.,* (1993) and Weykamp *et al.,* (1993) various techniques are used to measure HbA1c shows different values for haemoglobin variants and can cause some problems in monitoring and management of DM. So to overcome this problem, one should choose a method that meets the following conditions; haemoglobin variants should be separated and quantified reliably, and purpose should be specific to HbA1c with no cross-reactivity with other haemoglobin variants like HbA0 (Non-glycated fraction of HbA) and HbA0 (Glycated species of HbA). The Alere Afinion AS 100 HbA1c, Biohermes A1CEZ, ERBA glycohaemoglobin kit and BIO-RAD variant HbA1c kit (HPLC) are commercially available methods with clinically proven results, whereas our LFA is a newly developed prototype which requires further optimization to get excellent sensitivity and specificity.

CONCLUSION

Based on the results obtained so far through newly developed LFA, compared with gold standard and other optimized methods. The LFA has proved to be rapid, simple, inexpensive, the point of care method with excellent sensitivity and specificity.

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