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# Development and validation of an in-house handheld ELISA kit to detect glycosylated hemoglobin in human whole blood

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## ABSTRACT

**Background:** Diabetes mellitus (DM) is known as one the oldest disease known to a human being and it is defined as a group of metabolic disorders characterized by the presence of hyperglycemia. **Aims and Objectives:** The present study describes the development and evaluation of a cost-effective and straightforward in-house handheld enzyme-linked immunosorbent assay (ELISA) kit for the determination of HbA1c in human whole blood of diabetic patients and is suitable for resource-poor settings. **Materials and Methods:** A total of 1056 specimens were collected for assay validation purposes. To check the proficiency of our diagnostic strategy, we compared our test results with high-performance liquid chromatography (HPLC) and a commercially available ELISA kit for HbA1c. The Institutional Ethical Committee approved this study (IEC). SPSS 16 statistical software analyzed all the data. The correlation regression was done to compare the assay. The level of significance of this study was $P<0.001$. **Results:** The sensitivity, specificity, and efficiency of the in-house HbA1c ELISA kit range from 98.8%, 100%, and 99.24%. **Conclusion:** The newly developed in-house handheld HbA1c ELISA test kit is not only cost-effective, accurate, and straightforward but also gives good correlation with optimized methods such as HPLC and commercially available techniques in deciding the glycemic status of patients. Therefore, highly recommended for use in resource-poor settings in the management and diagnosis of DM.

**Key words:** Diabetes mellitus; Enzyme-linked immunosorbent assay; Glycated hemoglobin; High-performance liquid chromatography; International diabetes federation

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## INTRODUCTION

Diabetes mellitus (DM) is known as one the oldest disease known to a human being and it is defined as a group of metabolic disorders characterized by the presence of hyperglycemia, accompanied by a lesser or more significant abnormality in the metabolism of carbohydrates, lipids, and proteins, occurring due to the body does not produce insulin or insulin resistance or both. Chronic hyperglycemia in DM leads to the development of long-term complications affecting and damaging various organs such as blood vessels, eyes, kidneys, nerves, and heart.[1-3]

Globally, DM became one of the major health problems mainly affecting both developed and developing countries. The prevalence of DM was enormously increasing in low- and middle-income countries in Asia and Africa. According to the WHO report 2016, 422 million people are affected and living with DM globally.[4] The incidence of DM is rapidly increasing in developing countries, especially India. According to International Diabetes Federation in India 40 million people are affected and living with DM and the prevalence is projected to increase up to 70 million by 2020. India has a high prevalence of DM, so the WHO declared India as the diabetes capital of the world.[5-8]

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For better diagnosis and management of DM, various biochemical markers such as blood glucose, glycosylated hemoglobin (HbA1c), and C-peptide are estimated. At present a lot of treatment regimens are available, but regular monitoring is a big challenge for both physician and diabetic patients. Among the above biochemical markers, HbA1c is the most widely used marker for the diagnosis and management of DM. More recently the screening of HbA1c is used not only for monitoring and control of diabetes but also as a screening test in identifying people at future risk of developing the complications.[9-12]

HbA1c referred to substances that are formed by glucose binding to hemoglobin in the red blood cell. HbA1c is formed by a non-enzymatic reaction between plasma glucose and N-terminal valine of the β chain of hemoglobin, which is a two-step process. The initial step takes minutes to hours to form an aldimine complex (reversible reaction), followed by Amadori rearrangement (irreversible reaction) to create the stable ketoamine HbA1c. This glycation reaction will occur continuously and slowly over the lifespan of a red blood cell (120 days). The HbA1c values will provide glycemic history and also reflects the average blood glucose concentration over the entire lifespan of erythrocytes. Total glycated hemoglobin refers to all the glycated hemoglobins, such as HbA1, HbA1a1, HbA1b, and HbA1c. Among these fractions, a significant part was contributed by HbA1c.[13] HbA1c is usually determined by high-performance liquid chromatography (HPLC), borate affinity chromatography, and immunoassays. So to perform the above methodologies requires a well-trained technician and also requires expensive instruments with a long turnaround time. Even the WHO strategic advisory group of experts on *in vitro* diagnostics recommended using Handheld and point of care test kits for HbA1c for regular monitoring and diagnosis of DM.[14] As such, the present study describes the development and evaluation of a cost-effective and straightforward in-house handheld enzyme-linked immunosorbent assay (ELISA) kit for the determination of HbA1c in human whole blood of diabetic patients and is suitable for resource-poor settings.

### Aim

The aim of the present study is to develop rapid, user-friendly,specific and sensitive point of care diagnostic assay for detection of glycosylated haemoglobin (HbA1c) in whole blood samples.

### Objectives of the study

1 To develop, optimise, and validate a simple, rapid and in-house handheld ELISA kit for the determination of HbA1c in whole blood samples of diabetic patients and suitable for field condition.

## MATERIALS AND METHODS

### Participants and ethical consideration

The majority of this study was performed at MNR Medical College and Hospital, Sangareddy, Telangana. This study was approved by the Institutional Ethics Committee of MNR Medical College and Hospital, Sangareddy, Telangana State, India, conducted on November 8, 2016, Registration no.ECR/834/Inst/TG/2016. People who attended the diabetic clinic were involved in the study.

### Sample collections

A total of 1056 whole blood samples were collected in vacutainer with EDTA from people attending the diabetic clinic at MNR Medical College and Hospital, during the period from December 2016 to June 2018. 5 ml of whole blood was collected from the anti-cubital vein from each participant. Total 1056 samples were conformed with a standard gold method that is HPLC with different HbA1c concentrations from 4.5% to 14%.

### Preparation of reagents

All the reagents required for preparation of the coated ELISA plates and for carrying out the initial competitive ELISA test prepared before the beginning of the subsequent steps. These reagents included: Coating Buffer, Washing Buffer, Blocking Buffer, PBS, Stop Solution (0.5M Sulfuric acid). The appropriate reagents were weighed and dissolved in the proper media.

### Development of in-house ELISA kit

An *in vitro* quantitative ELISA to detect the amount of HbA1c in given human whole blood samples identified using a competitive ELISA methodology. The assay employs the competitive inhibition enzyme immunoassay technique. In brief, paired monoclonal antibodies, mab anti hemoglobin clone 6 and mab anti-HbA1c clone 16 (Arista Biologicals. Inc., USA) were selected to develop the competitive ELISA of which mab anti-HbA1c clone 16 is coated to the microwell plates to capture the specific HbA1c, and biotinylated detector monoclonal anti-hemoglobin clone 6 was used to detect the particular Hb1Ac (Figure 1). A concentration curve for the amount of antibody to be coated was optimized, and 135 ng per well was used to coat the plates for batch preparation. Microwell Polysorp plates (Nunc. Inc, Switzerland, Cat# 469957) were coated with the capture antibody at a concentration of 1.35 micrograms per ml in coating buffer overnight at 4° temperature, and the plates were washed with 1X PBS and were dried under the dehumidified condition and sealed in aluminum bags until further use. The whole blood of 1 ml was used in 100 ml of diluent buffer and loaded in each well and incubated for 1 h at room temperature. A competitive inhibition reaction was launched between biotin-labeled mab anti-Hb

clone 6 (reagent A) and unlabeled mab anti-HbA1c. After incubation, the unbound conjugate was washed off, and the next streptavidin-conjugated Horseradish Peroxidase (reagent B) is added to each microwell plate and incubated. The strips thus incubated were washed with 1X PBS 3 times for 5 min intervals and further developed using TMB (Arista Biologicals. Inc) substrate that has been stabilized to create chromogen in the reaction mixture. The resultant reaction was stopped by the addition of 1N sulfuric acid, and the quantitative chromogen was measured at 450 nm in a Handheld ELISA reader.

### Assay procedure

1. Add 50 ml of each dilution of standard, blank, and samples into appropriate wells. Before adding determine the wells for diluted standard, sample, and blank. Prepare 5 wells for standard points, 1 well for blank. And then add 50 ml of detection reagent A to each well immediately. Shake the plate gently and incubate for 1 h at 37°C.
2. Aspirate the solution and wash with 1X wash buffer (1XPBST) 3 times for 5 min intervals. After the last wash removes any remaining wash buffer by decanting or aspirating.
3. Now add 100 ml of detection reagent B (working solution) to each well. Incubate for 20–30 min at 37°C after covering the plate with a plate sealer.
4. Repeat the wash/aspiration process a total of 5 times as conducted in step 2.
5. Add 90 ml of substrate solution to each well. Incubate for 10–20 min at 37°C. Cover the plate with a plate sealer to protect from light. The liquid will turn blue with the addition of substrate solution.
6. Add 50 ml of stop solution to each well. The liquid will turn yellow with the addition of a stop solution.
7. Read the optical density (OD) at 450 nm immediately by using a microplate reader or ELISA reader.

The summary of the above procedure was explained in the schematic diagram (Figure 2).

### Standardization and optimization of ELISA system

Calibration curves, as stipulated by Findlay 2006, form a critical component of the preparation of ELISA kits and the use of at least six known concentrations of analytes highly recommended.[15] Jack (1995) suggests that samples dilutions that are to be used to prepare the calibration curve provide signals that fall in the area of 50% B/B0 wherein B=y−b, B0=y0−b, y0=the maximum signal, and b is the selected point.[16]

### Preparation of a standard curve

Determine the concentration of a purified HbA1c. Dilute the HbA1c serially to the level of the standard is 3000 μg/ml first dilute the stock solution to 1000 μg/ml.

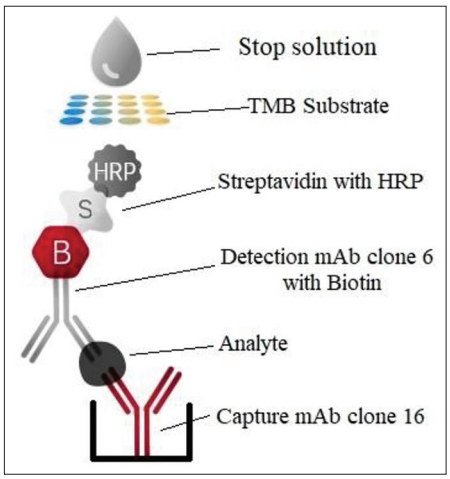


**Figure 1:** Schematic representation for the detection of HbA1c using competitive ELISA

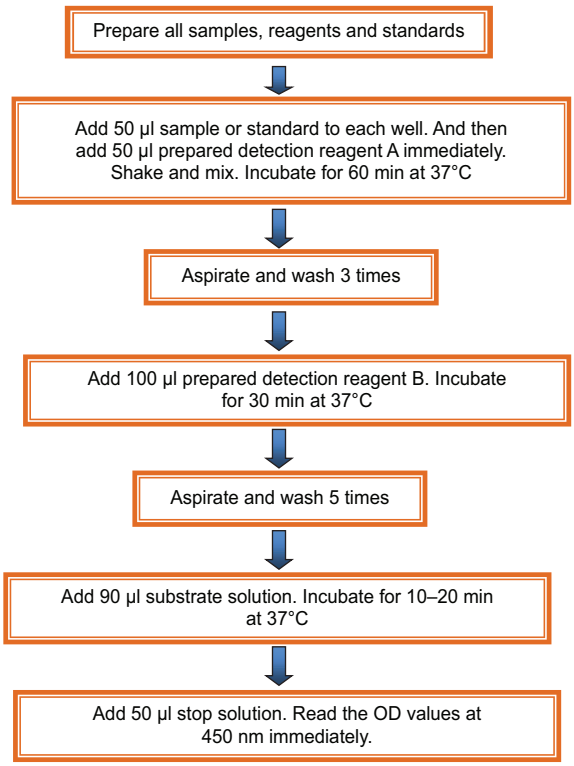


**Figure 2:** Schematic representation of assay procedure for the detection of HbA1c using competitive ELISA

Then prepare five tubes containing 0.6 ml standard diluents and produce a triple dilution series as shown in Figure 3. Mix each Eppendorf tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 1000 μg/ml, 333.33 μg/ml, 111.11 μg/ml, 37.04 μg/ml, and 12.35 μg/ml and the last Eppendorf tubes with standard diluents are blank as 0 μg/ml. Carry out an ELISA test in duplicate using the dilutions as the samples. Plot a curve of HbA1c concentration for each dilution on the Y-axis against its OD 450 nm reading on the X-axis. Use

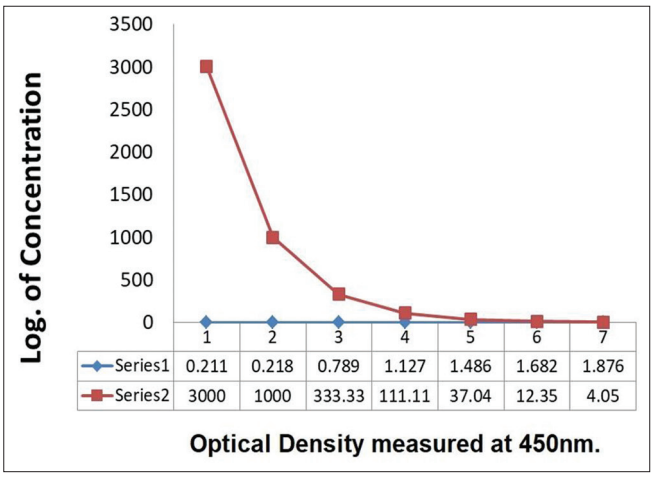


**Figure 3:** Standard curve for in-house HbA1c ELISA test kit

the graph to obtain HbA1c levels for different samples from OD 450 nm readings after carrying out an ELISA test.

### Performance of ELISA for estimation of HbA1c

#### *Assay accuracy*

Accuracy is defined as the closeness of the test results obtained by the method to the real value derived from the reference standard. Two rules were made and used to check the accuracy of the In-house ELISA kit for HbA1c:

a) The correlation coefficient was determined by linear regression analysis to verify the reproducibility of HbA1c% values obtained by a standard gold method HPLC on an in-house ELISA assay.
b) The degree of association between two methods to categorize patients according to glycemic control (controlled [HbA1c<6.5%], uncontrolled [HbA1c>6.5%]). The cutoff was based on recent recommendations made by American Diabetes Association (ADA 2009) (i.e., controlled diabetes [HbA1c <6.5%] and uncontrolled diabetes [HbA1c>6.5%]). The sensitivity and specificity were estimated based on the values obtained from the standard gold method and in-house ELISA test.

#### *Diagnostic sensitivity and specificity*

A set of 1056 samples was used for the determination of diagnostic sensitivity and specificity. These samples were analyzed using the kit developed in this project, and HPLC (Bio-Rad D-10 for HbA1c) to determine comparative performance. The HPLC method (Bio-Rad D-10) was used as the comparative standard gold method. The contingency table was prepared to help in the calculation of diagnostic sensitivity and specificity using the following formula described by Crowther, (2001)[17] and Mc NEMAR's test was used for Chi-square and P-value. Diagnostic Sensitivity % = True positives (TP)/True positives (TP)+False Negatives (FN)×100; Diagnostic Specificity % = TN/FP+TN×100; Positive Predictive Value (PPV) % = TP/TP+TP×100; Negative Predictive Value (NPV) % = TN/TN+FN×100; and Diagnostic Efficiency % = TP+TN/TP+FN+TN+FP×100.[18,19]

### Comparison of in-house HbA1c ELISA kit with commercial HbA1c ELISA kit

The correlation coefficient was determined by linear regression analysis to check the reproducibility of HbA1c% values obtained by a commercially available ELISA kit on an in-house ELISA assay.

### Commercial ELISA method

The antigen to HbA1c was detected by commercial ELISA method from the Cloud-Clone Corporation, Katy, Texas, USA with 1056 HPLC tested samples. The test was performed according to the manufacturer's instructions and each ELISA test was valid only if the relevant controls (negative, positive, and cutoff controls) were within the range as described by the manufacturer.

### Comparison of in-house ELISA kit with Handheld ELISA reader versus clinically optimized ELISA reader

The correlation coefficient was determined by linear regression analysis to check the reproducibility of HbA1c% values obtained by a Handheld ELISA reader with an existing clinically optimized ELISA reader.

### Statistical analysis

An OD value of the in-house ELISA was analyzed compared with OD values of the commercially available ELISA kit by Pearson correlation coefficient. SPSS version 16; Mc NEMAR's test and M.S. Excel 2010, used for statistical calculations.

## RESULTS

### Preparation of a standard curve

HbA1c levels quantitatively estimated in whole blood samples by plotting a calibration curve between the log of the concentration of the standard on the Y-axis and OD values of the standard on the X-axis (Figure 3).

### Samples were used for validation of the HbA1c ELISA kit

A total of 1056 subjects were involved in this study. The whole blood samples of 1056 were analyzed for validation of the HbA1c ELISA kit. According to the recommendations made by ADA (ADA, 2009), the study subjects were categorized into controlled DM (≤6.5/HbA1c %) and uncontrolled DM (≥6.5 HbA1c %). Based on the ADA recommendations the 1056 whole blood samples were divided into two groups, 378 controls (non-diabetic) and 678 uncontrolled (diabetic) with the age range from 35 to 65 years. Out of 378 (35.79%) non-diabetic controls, 229 (21.68%) were males and 149 females (14.10%), and

in 678 (64.20%) diabetic cases, 401 (37.97%) were males and 277 (26.23%) females (Table 1 and Figure 4).

### Assay accuracy

HbA1c results measured by in-house ELISA and HPLC (Bio-Rad D-10) method compared for 1056 samples and correlations coefficient between HbA1c results obtained by two techniques were determined that exhibited good correlation, (r=0.960, P<0.001, y=−0.464+0.954x) (Figure 5).

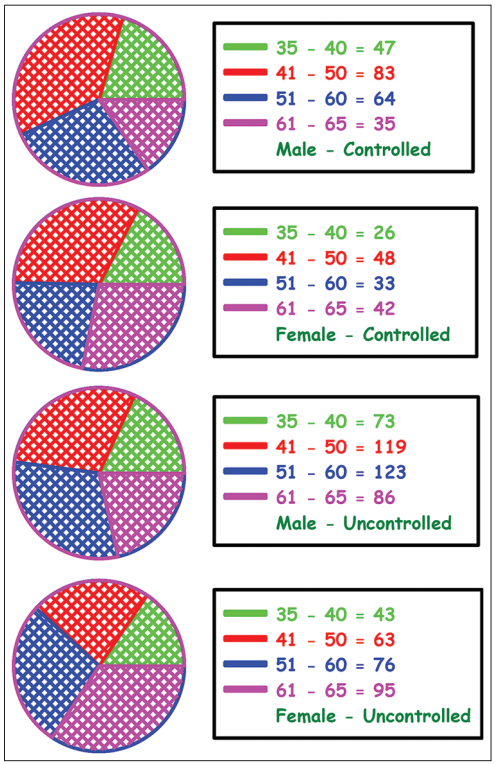


**Figure 4:** The association of age and gender among controlled and uncontrolled diabetics (n=1056). $\chi^2$=34.014; P<0.001

### Diagnostic sensitivity and specificity

A set of 1056 samples was picked for the determination of diagnostic sensitivity and specificity. These samples were analyzed using the kit developed in this project, and HPLC (Bio-Rad D-10) to determine comparative performance. The HPLC (Bio-Rad D-10) was used as the comparative Gold Standard. The Contingency table was prepared to help in the calculation of diagnostic sensitivity and specificity using the following formula described by Crowther, (2001a) and Mc NEMAR's test: The kit under development showed that 670 samples were uncontrolled DM and 378 samples controlled DM. The HPLC (Bio-Rad D-10) showed that 678 samples were uncontrolled DM and 378 controlled DM.

The In-house ELISA test showed a total of 670 uncontrolled DM cases out of 678 with the HPLC (Bio-Rad D-10) while 8 of the negative in-house ELISA tests were positive with HPLC thereby showing a diagnostic sensitivity of 98.82% and a specificity of 100%. The PPV was 100%, and the NPV was 97.92%. Overall the efficiency of the in-house ELISA test kit was 99.24%, respectively (Table 2).

### Comparison of in-house ELISA kit with commercial available HbA1c ELISA kit

The correlation coefficient was determined by linear regression analysis to check the reproducibility of HbA1c% values obtained by a commercially available ELISA kit

**Table 1: Age and gender distribution of controlled and uncontrolled diabetics**

| Age (Years) | Controlled (Non-Diabetic) (n=378) and (35.79%) | | Uncontrolled (Diabetic) (n=678) and (64.20%) | |
|---|---|---|---|---|
| | Males (n=229) (21.68%) | Females (n=149) (14.10%) | Males (n=401) (37.97%) | Females (n=277) (26.23%) |
| 35–40 | 47 (4.45%) | 26 (2.46%) | 73 (6.91%) | 43 (4.07%) |
| 41–50 | 83 (7.85%) | 48 (4.54%) | 119 (11.26%) | 63 (5.96%) |
| 51–60 | 64 (6.06%) | 33 (3.12%) | 123 (11.64%) | 76 (7.19%) |
| 61–65 | 35 (3.31%) | 42 (3.97%) | 86 (8.14%) | 95 (8.99%) |
| Chi-square ($\chi^2$), P-value | $\chi^2$=9.427 and P<0.024 | | $\chi^2$=14.339 and P<0.002 | |
| | $\chi^2$=34.014 and P<0.001 | | | |

**Table 2: Specificity and sensitivity of in-house ELISA kit in comparison with HPLC**

| | | | Sensitivity (%) | Specificity (%) | NPV (%) | PPV (%) | Efficiency (%) | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| HPLC | Uncontrolled DM | 678 | 100% | 100% | 100% | 100% | 100% | 0 | 1 |
| | Controlled DM | 378 | | | | | | | |
| | Total | 1056 | | | | | | | |
| In-house ELISA | Uncontrolled DM | 670 | 98.82% | 100% | 97.9% | 100% | 99.24% | 6.125 | <0.013 |
| | Controlled DM | 378 | | | | | | | |
| | Total | 1048 | | | | | | | |

HPLC: High-performance liquid chromatography, ELISA: Enzyme-linked immunosorbent assay, NPV: Negative predictive value, PPV: Positive predictive value

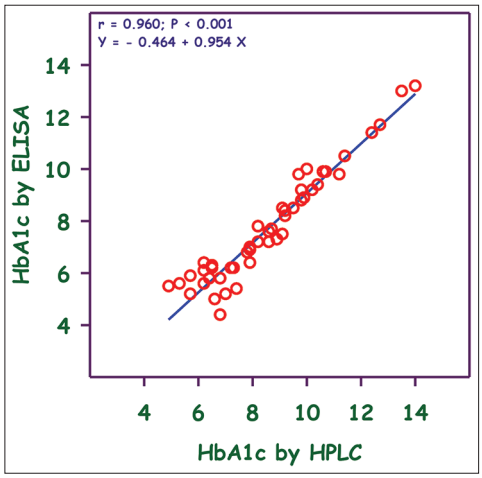


**Figure 5:** Comparison of HbA1c results measured by in-house HbA1c ELISA test kit and HPLC (Bio-Rad variant HbA1c)

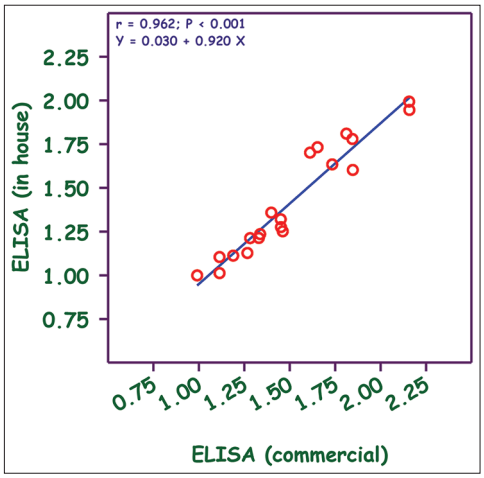


**Figure 6:** Comparison of in-house HbA1c ELISA test kit with commercial HbA1c ELISA kit

on an in-house ELISA assay. The developer kit has a perfect Pearson correlation with the commercial ELISA kit for HbA1c and correlation of (r=0.962, p<0.001, y=0.030+0.920x) with the commercial ELISA kit, signifying significant correlation (Figure 6).

### Comparison of in-house ELISA kit with Handheld ELISA reader versus clinically optimized ELISA reader

The correlation coefficient was determined by linear regression analysis to check the reproducibility of HbA1c% values obtained by in-house ELISA assay with Handheld ELISA reader and clinically optimized ELISA reader. The developed in-house ELISA kit had a perfect Pearson correlation with the handheld ELISA reader and optimized and existing clinically optimized ELISA reader. The correlation of (r=0.991, P<0.001, y=0.113+0.889x) signifying significant correlation between two ELISA readers (Figure 7).

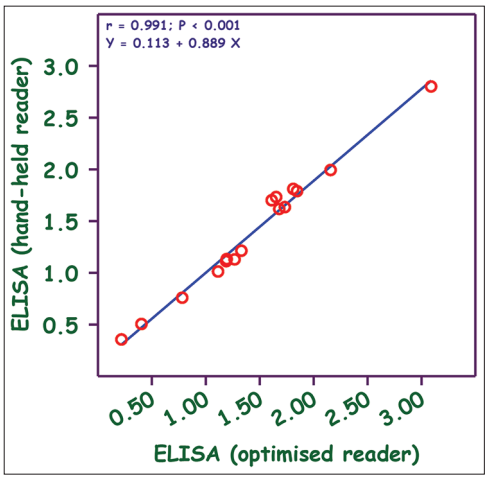


**Figure 7:** Comparison of in-house HbA1c ELISA test kit with Handheld ELISA reader versus clinically optimized ELISA reader

## DISCUSSION

Diabetic care depends on continuous monitoring and maintaining blood glucose levels for the long term. Many point-of-care tests have been developed for blood glucose monitoring. However, the blood glucose levels only reflect the present condition of patients. This is inadequate in assessing glucose regulation. So HbA1c has become the most important indicator of overall glucose level in a patient during 120 days.[20,21] For the last two decades, HbA1c choose as the best marker used for long-term monitoring and diagnosis of DM. Globally, The International Federation of Clinical Chemistry and Laboratory Medicine and the international diabetic association stated that pure HbA1c should use as one numerical value and one unit. At present irrespective of the statistical unit used for hemoglobin assay, the concept of long-term monitoring of diabetes is dependent on the efficiency or accuracy of the HbA1c assay itself.[22]

In the present study, 1056 whole blood samples are collected and used for the validation of an in-house HbA1c ELISA test kit with the standard gold method HPLC. Based on the ADA recommendations, the 1056 whole blood samples were divided into two groups, 378 controls (non-diabetic) and 678 uncontrolled (diabetic) within the age range from 35 to 65 years. Out of 378 (35.79%) non-diabetic controls, 229 (21.68%) were males and 149 females (14.10%), and in 678 (64.20%) diabetic cases, 401(37.97%) were males and 277 (26.23%) females as shown in Table 1 and Figure 4. If you compare the males and females among the controlled group, there was a significant difference (P<0.024) with a Chi-square value of $\chi^2$=9.427. If you compare the males and females among the uncontrolled (Table 1), there was a significant difference (P<0.002) with a Chi-square value

of $\chi^2$=14.339. Overall if you compare the association of controlled and uncontrolled DM irrespective of age and gender there was a significant difference (P<0.001) with a Chi-square value of $\chi^2$=34.014. If you observe the uncontrolled group, more number of diabetics is observed in the age group of 41–60. This may be due to the incidence of getting DM is increased along with age and gender. With the increase of age, many changes were observed in the human body, such as the pancreatic function is gradually declined, receptor activity of insulin, and tissue sensitivity is declined along with the age. So this may be the reason to have more diabetic patients in the 41–60 age groups (Table 1 and Figure 4). A similar study was reported by Ma et al., 2016.[23]

In the present study, newly developed in-house, ELISA for HbA1c got good correlation with standard gold method HPLC (r=0.960, P<0.001, y=−0.464+0.954x) (Figure 5). It supports the proper standardization and uniformity of this method to other immunoassays indicated in other studies.[24] According to ADA, the therapeutic goals for the treatment of patients with diabetes are ≥6.5%.[25] A particular HbA1c method despite showing excellent statistically significant correlation might differ in the categorization of the status of glycemic control of individual patients.[26] This misclassification, in turn, may lead to erroneous decisions for treatment requirements or modifications.[26] In this study, both HPLC and in-house ELISA classified most of the cases in the same category of controlled (<6.5%) or uncontrolled diabetes (>6.5%). The only misclassified cases by the in-house ELISA method were 08 (1.17%). The developer kit was found to have a diagnostic sensitivity of 98.92%, diagnostic specificity of 100%, PPV 100%, NPV 97.92%, and overall efficiency value of the in-house ELISA test kit is 99.24% with an established HPLC (Bio-Rad D-10) kit as a gold standard (Table 2). Among clinical laboratories different performance properties predict the most valuable information; hence, the PPV and NPV are, more informative than sensitivity and specificity. The estimated PPV and NPV values for the in-house hba1c assay were above 95%.

In the present study, a newly developed in-house HbA1c ELISA test kit correlated with a commercially available ELISA kit (cloud-Clone Corporation, Katy, Texas, USA). The developed in-house kit has a perfect Pearson correlation with the commercial ELISA kit for HbA1c and correlation of (r=0.962, P<0.001, y=0.030+0.920x) with the commercial ELISA kit, signifying significant correlation (Figure 6). Based on the result obtained through linear regression in-house HbA1c ELISA kit was cheaper when compared with the imported commercially available ELISA kit and an important note is the commercially available ELISA test kit is manufactured only for the research purpose (Table 3).

Globally, no existing diagnosing lab is utilizing the ELISA method for the determination of HbA1c. Hence, we developed an in-house ELISA kit of eight well strips, suitable for handheld ELISA readers. This is not similar to the existing ELISA reader used at present in the clinical laboratory. It can use as a point-of-care kit in resource-limited settings. To validate newly developed eight well handheld ELISA kits also took linear regression analysis to check the reproducibility of HbA1c% values obtained by in-house ELISA assay with Handheld ELISA reader and clinically optimized ELISA reader. The developed in-house ELISA kit had a perfect Pearson correlation with the handheld ELISA reader and existing clinically optimized ELISA reader. The correlation of (r=0.991, P<0.001, y=0.113+0.889x) signifying significant correlation between two ELISA readers (Figure 7).

At the end of the study, we report that dependence on the correlation or statistical significance of linear regression between the reference HPLC method and alternative methods is baseless unless it correctly classifies patients based on their glycemic control. Our in-house HbA1c ELISA method for estimation of HbA1c is superior to other assays with 99.24% accuracy to categorize individual patients according to their glycemic control, which is of vital significance in deciding treatment requirements.

**Table 3: Comparison of in-house ELISA kit with commercial ELISA kit**

| Parameters | Commercial ELISA kit | In-house ELISA kit |
|---|---|---|
| Type of sample | Serum, plasma, and erythrocyte lysates | Serum, plasma, and erythrocyte lysates |
| Sample pretreatment | Required | Required |
| Sample dilution | Required | Required |
| Sample volume | 50–100 ml | 50 ml |
| Time for result | 60–120 min | 60–120 min |
| Skill level | High | Low |
| Equipment | ELISA instrument | Handheld ELISA instrument |
| Power | Instrument requires power 110 or 220 | Instrument can work on four battery cells |
| Storage temperature | −20°C | −20°C |
| Cost/test | High | Low |

ELISA: Enzyme-linked immunosorbent assay

### Limitations of the study

We performed the study with limited number of samples, which is not enough to establish our diagnostic strategies in laboratory medicine. Further study with large number of samples required to evaluate the HbA1C ELISA KIT combined with Hand-Held ELISA reader.

## CONCLUSION

The newly developed in-house handheld HbA1c ELISA test kit is not only cost-effective, accurate, and straightforward but also gives good correlation with optimized methods such as HPLC and commercially available techniques in deciding the glycemic status of patients. Therefore, highly recommended for use in resource-poor settings in the management and diagnosis of DM.

## ACKNOWLEDGMENTS

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**NP -** Concept and design of the study, prepared first draft of manuscript; **PCM -** Interpreted the results; reviewed the literature and manuscript preparation; **RKBN -** Co-guide; Statistical analysis; **MNK -** Sample collection for evaluation of ELISA kits; **RGP -** Guide, Concept, coordination, statistical analysis and interpretation, preparation of manuscript and revision of the manuscript.

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