Research Article

Evaluation of an Enzymatic Hemoglobin A1C Assay

Numan Majeed,[1] Fatima Kanani,[2] Adnan Mustafa Zubairi[3]

[1-3]*Indus Hospital and Health Network, Karachi, Pakistan*

Abstract

Background: Different modalities have been designed for estimation of HbA1c, that include ion-exchange High Performance Liquid Chromatography (HPLC), immunoassay, enzymatic assays and capillary electrophoresis. Although based on different principles, these analytical processes have to exhibit robust performance so that an overall comparability of assay values can be ensured which are traceable to the available reference method.

Objectives: Objective of the study was to evaluate HbA1c measurement on Abbott Alinity C system against ion exchange high performance liquid chromatography.

Methods: We performed different experiments to evaluate measurement of HbA1c on Alinity C system by Abbott. These studies included accuracy, precision, linearity, carry over and method comparison in patients without any hemoglobin variants. Evaluation Protocol (EP) evaluator was used for data analysis.

Results: All the experiments passed the minimum passing criteria. All the claims by the vendor were successfully verified with and addition that vendor claims 12-hour stability while our study showed a stability of 18 hours. In precision the coefficient of variance in our experiment showed better results.

Conclusion: Alinity C system can be used reliably and efficiently for the assessment of HbA1c in patients without haemoglobin variants as our study did not include samples with variant hemoglobin.

Received: 14-11-2023 | **Revision:** 20-04-2024 | **Accepted:** 05-06-2024

Corresponding Author | Dr. Numan Majeed, Resident, Indus Hospital and Health Network, Karachi, Pakistan

Email: numan.mjd@gmail.com

Keywords: Glycated hemoglobin, Alinity C, HPLC, method validation

Introduction

Diabetes mellitus is one of the fastest growing non communicable epidemic, affecting millions of people globally, which affects quality of life by its many complications[1] such as nephropathy, neuropathy, retinopathy.[2] According to an estimate about 50% cases of diabetes are yet undiagnosed, particularly in areas that are remote and difficult to access.[3] Accurate and prompt diagnostic testing, which is often done by hospital clinical laboratories using a variety of techniques, is necessary for the diagnosis and efficient treatment of diabetic patients.[4,5]

Glycated hemoglobin (HbA1c) is an important marker of mean glycemic index of diabetic patients that can be used for monitoring purposes and is a strong predictive marker for complications of diabetes.[5,6] HbA1c is also included in the diagnosis of diabetes by the American Diabetes Association.[7] Several methods have been developed for quantification of HbA1c including ion-

Production and Hosting by KEMU

https://doi.org/10.21649/akemu.v30i2.5567

2079-7192/

exchange High Performance Liquid Chromatography (HPLC), immunoassay, enzymatic assays and capillary electrophoresis, cation exchange high performance liquid chromatography.[8] Some kits/assays designed to estimate HbA1c levels may report false results due to presence of hemoglobin variants such as HbS, HbE, HbF and HbD. These interferences may result in clinically significant variations resulting in either over treatment and hypoglycemia or under-treatment leading to hyperglycemia, both of which are potentially detrimental to human health.[4]

To identify the fraction of hemoglobin A that is glycated at one or both of the N-terminal valines of the beta-chain, Abbott Diagnostics has developed an automated whole blood Hemoglobin A1c (HbA1c) test on the Alinity c system. The N-terminal fructosyl dipeptides of the beta-chain of HbA1c are specifically measured using an enzyme approach that automatically lyses red blood cells. Glycated hemoglobin (HbA1c) and total hemoglobin (THb)are the two distinct measures, that are utilized to calculate the percent HbA1c and (National Glycohemoglobin Standardization Program [NGSP] units(mmol/mol).[9]

Although based on different principles, these analytical processes have to exhibit robust performance so that an overall comparability of assay values can be ensured which are traceable to the available reference method.[10]

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Task Force on Implementation of HbA1c standardization (TF-HbA1c) has advocated the use of sigma metrics for the purpose of evaluation and setting the quality targets of glycated HbA1c.[11] In the laboratory this metrics is a quality indicator that provides a benchmark for process performances. It makes sure that the analytical characteristics such as precision and bias are within the total allowable error.[4]

The assay is certified by NGSP, standardized to International Federation of Clinical Chemistry (IFCC), and traceable to the Diabetes Control and Complications Trial (DCCT).

Methods

Alinity C system is a standalone as well as an integrated platform in total lab automation systems. It is based on photometric and turbidimetric methods. HbA1c is being performed by enzymatic method. The reagent is provided as ready for use cassettes with an on-board stability of 30 days.

Various experiments were carried out for the purpose of evaluation, which are summarized below

1. Clinical & Laboratory Standards Institute provided Evaluation Protocol no. 15-A3 (CLSI EP15-A3)[(12)] was followed for precision. Intra and inter day precision were assessed by analyzing three patient pools (with values of 5.62%, 10.2% and 13.9%) for a period of 5 days with series of five replicates each day.[4]
2. Accuracy was analyzed using proficiency Testing Surveys (GH5I-A 2022) provided by College of American Pathologists at various concentrations.

   Whole blood received (in EDTA containing, vacuum tubes, by Becton, Dickinson Vacutainer) were analyzed in the laboratory for HbA1c on the current method (HPLC) on Bio-Rad Turbo Variant II and Alinity c for HbA1c samples without haemoglobin variants across the analytical measuring range. The samples were taken from hematology department that came for Hb variant studies and were found not having any variant.
3. Two experiments were conducted for method comparison:
   a. 80 samples with HbA1c levels within analytical measurement range and Hemoglobin levels between 12 and 16 mg/dL were analyzed by both methods. Specimens were categorized on HbA1c levels as follows:[13-15]
      - 4.0 -5.7%
      - >5.7, <7.1%
      - 7.1-<10%
      - 10.0-14%

   b. 20 specimens with hemoglobin levels below 7g/dL and not demonstrating any variant hemoglobin were used to compare the methods at very low levels of Hemoglobin.
4. Linearity was done following CLSI EP06Ed2,[16] by measuring five HbA1c levels in duplicate covering the entire AMR and using CAP linearity material (Hemoglobin A1c Calibration Verification/ Linearity LN15-B 2020).

5. Carryover was assessed according to CLSI EP10-A3 (17) using samples with low, mid, and high concentrations of HbA1c (4.9, 7.0, and 9.6%, respectively) run in the order M-H-L-M-M-L-L-H-H-M, where L is low concentration, M is mid-level and H is high level
6. CLSI EP35 was used to perform stability experiment. Samples of 5 patients were taken. Each sample was run in duplicate at six-time intervals without mixing according to following grid:
   - (time from draw) 0 hours, 1 hour, 4-hour, 8-hour, 12 hours, 24 hours). The samples were stored at room temperature (24-26OC).

Total allowable error (TE_a) was kept at 6%, which has been set by College of American Pathology (CAP)

EP Evaluator was used for data analysis. Statistical analysis/ experiment passing criteria as mentioned in respective CLSI guidelines is shown in Table 1.

Results

The precision results passed at all three levels. The coefficient of variance (CV) at all the three levels was found to be <2%.

- At 5.62%
  - Between run CV was 0.7 which was exactly the same as that claimed by Abbott.
  - Between day CV was 0.0, no claim made by Abbott
- At 10.20%
  - Between run CV was 0.1, while that claimed by Abbott is 0.4.
  - Between day CV was 0.5, no claim made by Abbott
- At 13.90%
  - Between run CV was 0.1, while that claimed by Abbott is 0.3.
  - Between day CV was 0.0, no claim made by Abbott

We compared Alinity C system with Bio-Rad Variant II for normal (non-anemic) and anemic samples. All the samples were tested for variants and only those were selected which had no haemoglobin variants. All the experiments intercept and slope both are in allowable limits hence experiment was considered pass.

Table 2 shows comparison of proficiency testing samples run on the instrument with the assigned target values.

All the samples were within the allowable limits hence the experiment was declared pass.

The prescribed linearity of the instrument is 12.8. All the samples (CAP linearity material) were within the specified range. Slope was found to be 1.012 (0.996 to 1.027) while intercept to be 0.10 (-0.23 to 0.02), which fall within the allowable limits declaring experiment as pass. Table 4 shows results of the linearity study.

Table 2: *Results of accuracy experiment*

Sample No	Target		Measured Values		
	Run 1	Run 2	Range	Midpoint	Accuracy
1 (GH51-01)	7.1	7.1	6.4 to 7.7	7.1	Pass
2 (GH51-02)	5.6	5.6	5.2 to 6.1	5.6	Pass
3 (GH51-03)	6.1	6	5.5 to 6.6	6	Pass
4 (GH51-04)	12.1	12.1	11 to 12.6	11.8	Pass
5 (GH51-05)	6.4	6.5	6 to 7	6.5	Pass

Table 1: *Passing criteria for each experiment*

No.	Experiment	Criteria	Range of acceptability		
1.	Linearity	Non-linearity: <2%	TEa: 6%, % of nonlinearity: 25%		
2.	Accuracy	Midpoints of the target ranges for the lowest and highest specimens respectively are within proximity limits of the Reportable Range Limits, and 2) these two specimens also pass accuracy		**Reportable range**	**Proximity limits**
			Low	4	3.6 to 4.4 %
			High	14	12.6 to 15.4 %
3.	Precision	SD Goal is within the 95% confidence limit	Manufacturer claim, given in package insert.		
4.	Carry over	Carryover is less than the Error Limit (0.1%)	Error limit 6%		
5.	Method comparison	The slope is 1.00 (within 95% confidence) The intercept is 0.00 (within 95% confidence)	TEa:6%		

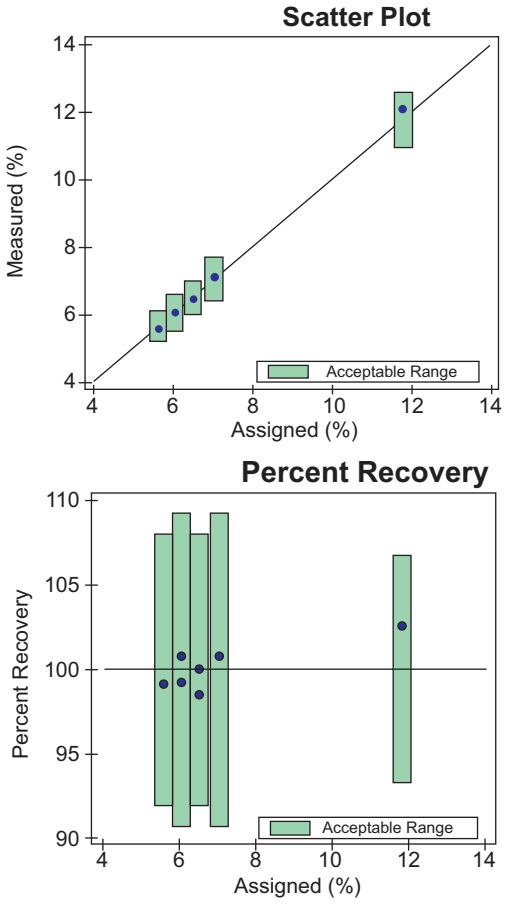

Figure 1: *Scatter plot and recovery chart of the accuracy study*

Method comparison was done at two levels, i.e. hemoglobin levels with in normal reference range and in anemic range. Table 3 shows regression (both Deming and regular) for the both anemic and nonanemic samples. The linearity experiment showed liner results (pass) in table 4

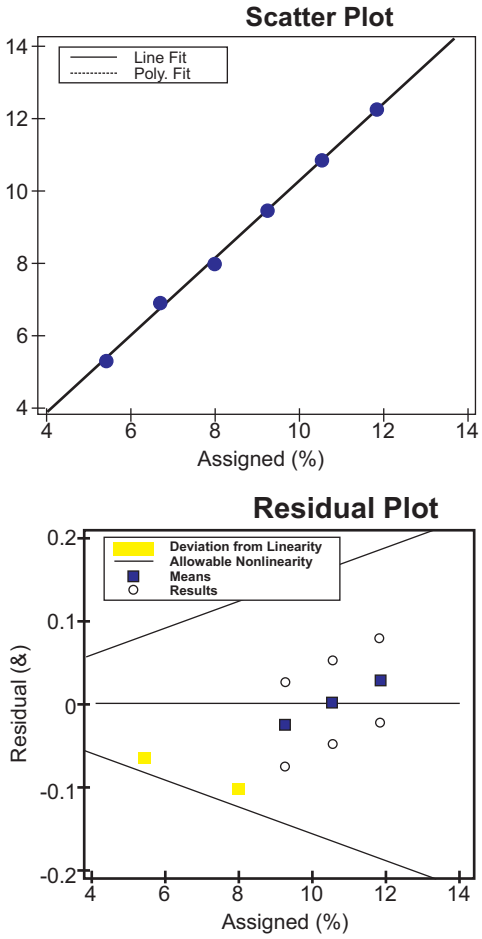

Figure 2: *Scatter plot and residual plot for the Linearity study*

The carry over experiment was declared pass with a total carryover effect of -0.2%.

All the samples were run at specified intervals without

Table 3: *Regression analysis for anemic and non-anemic samples*

	Non-Anemic		Anemic	
	Deming	**Regular**	**Deming**	**Regular**
Slope	1.030 (1.017 to 1.043)	1.026 (1.013 to 1.040)	0.979 (0.954 to 1.004)	0.976 (0.952 to 1.001)
Intercept	-0.35 (-0.46 to -0.25)	-0.32 (-0.43 to -0.22)	0.10 (-0.03 to 0.23)	0.12 (-0.01 to 0.25)
Std Err Est	0.20	0.20	0.07	0.07

Table 4: *Linearity study results*

Sample	Assigned value	Mean	Poly Fit	Line Fit	Deviation from Linearity*	Deviation**
1 (LN15-07)	5.4	5.30	5.36	5.36	0.00	0.00
2 (LN15-08)	6.69	6.90	6.74	6.74	0.00	0.00
3 (LN15-09)	7.97	8.00	8.10	8.10	0.00	0.00
4 (LN15-10)	9.26	9.45	9.47	9.47	0.00	0.00
5 (LN15-11)	10.55	10.85	10.85	10.85	0.00	0.00
6 (LN15-12)	11.84	12.25	12.22	12.22	0.00	0.00

: Deviation from linearity: Deviation from linearity curve* *: Deviation: Deviation from assigned value*

mixing/shaking and the stability was found out to be 13.2 hours, which is more than the stability claimed by the manufacturer. (figure 3)

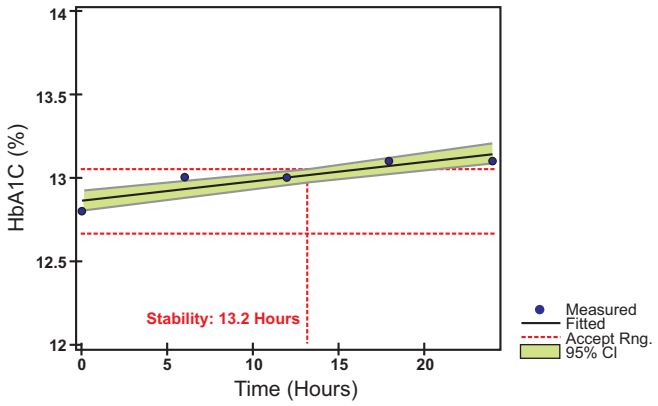

Figure 3: *Stability study cut-point*

Discussion

Estimation of HbA1c is an essential investigation for the diagnosis as well as monitoring of diabetes mellitus as per international guidelines including American Diabetes Association (ADA) and National Institute for Health and Care Excellence (NICE).[18] For the estimation of HbA1c levels, different methods and instruments have been developed. IFCC and NGSP has set specific analytical pre-requisites for and instrument to be deemed fit for clinically acceptable results of HbA1c.[18] Moreover, in the diagnosis of DM, HbA1c testing offers significant advantages over fasting plasma glucose. HbA1c is stable in blood samples, does not require fasting, has less variability within individuals, and is not affected by acute situations. Due to its critical clinical role, there is a global consensus that HbA1c results should be standardized. To achieve this, the IFCC has defined an internationally recognized HbA1c reference measurement system (RMS), ensuring the global interchangeability of result interpretation, independent of the assay used. Manufacturers of in vitro diagnostic systems are required to design commercial systems that meet the traceability requirements to this RMS.[19] Additionally, laboratory professionals must verify the alignment process to higher-order references and ensure that the marketed measuring systems' performance is appropriate for clinical use.[20]

Labs must adhere to a program of analytic performance control (CV <2%) applied to the method used to measure HbA1c in order to ensure the quality and clinical reliability of results produced. This program needs to be accredited by the NGSP or the IFCC.[21]

Our study demonstrated that maximum imprecision (CV=0.7%), the imprecision was higher at low concentration but improved as the value of HbA1c got higher till its linearity (13%).

The TOP-HOLE study is the one of the very few studies so far that performed validation of Alinity C system. But unlike our study in which we compared HPLC using Bir-Rad Variant II Turbo, the TOP-HOLE study used enzymatic Architect C-4000 with Alinity c stand-alone (S-A) system. Both of the instruments use same method and reagents, yet demonstrated a bias of -3.1at low level (31.9 mmol/mol equaling to 5.1%) and 4.59 higher level (87.2 mmol/mol equaling to 10.1% using control material.[19] But as both the platforms had same reagent and method, we cannot suggest which one is more reliable. Another study reported a total imprecision at different concentration levels ranging from 0.5-1.3%.[9]

Most of the studies conducted on enzymatic assay for estimation of HbA1c have used used other instruments as Alinity C system is comparatively newer and very few studies so far has been done. A study done by Monaco et al on the Abbott Architect system, a system that utilizes same reagent has shown very similar results as ours, our results showed a better agreement as compared to that shown in this study.[22] Similarly, another study by Sriwimol et al done on same principal, using Mindray, similar results were obtained.[23] Chalia et al also showed similar findings, they compared three systems, enzymatic on Abbott Architect, Roche turbidimetric and HPLC showing strong correlation with all the methods.[21] On Siemens Dimension (also an enzymatic assay) similar results were reported but the study also suggested that Dimension is not as cost effective as Biorad.[24]

Similarly, our study showed that the samples are stable for a period of 13.2 hour at room temperature and can be analyzed even without mixing and stirring. It is worthwhile to mention that Abbott in their kit literature mentions 8 hours of stability and recommends mixing of all samples before analysis. Although we did not use the total lab automation TLA) track system but it can decrease the turnaround time (TAT), as reported in the TOP-HOLE study to 1 hour only.

As Alinity system by Abbott is comparatively new, so as of now on Alinity systems very few studies have been done for validation of HbA1c enzymatic assay. Most of the studies done were by comparing Alinity with other enzymatic or immunoturbidimetric methods only one study reported comparison with HPLC, but it did not fulfil the CLSI recommended experiments criteria (Berman et al).

The major limitation of the study was that the samples were not run on TLA track system and TAT was not calculated. Secondly effect of various haemoglobin variants on HbA1c could not be verified because of lack of availability of such samples in sufficient numbers at various percentages.

Our study further opens the doors for further studies using more robust study on impact of variants as well as track-based time effectiveness and most of all cost calculations.

Conclusion

Abbot Alinity C system which is based on enzymatic estimation of HbA1c has passed the five experiments recommended by Clinical & Laboratory Standards Institute that include linearity, accuracy, precession, method comparison (both with normal and low level hemoglobin) and carry over experiments following their recommended protocols, hence it can be used reliably for estimation of samples without any hemoglobin variant.

Ethical Approval: The Institutional Review Board of Indus Hospital & Health Network, Karachi approved the study vide No. IHHN-IRB# IHHN_IRB_2022_09_027.

Conflict of Interest: The authors declare no conflict of interest.

Funding Source: None

Authors' Contribution:

NM: Conception & design, analysis & interpretation of data, drafting of article

FK: Conception & design, critical revision for important intellectual content, final approval

AMZ: Conception & design, final approval

References

1. Teodoro-Morrison T, Janssen MJ, Mols J, Hendrickx BH, Velmans MH, Lotz J, et al. Evaluation of a next generation direct whole blood enzymatic assay for hemoglobin A1c on the ARCHITECT c8000 chemistry system. Clin Chem Lab Med. 2015;53(1):125-32. doi: 10.1515/cclm-2014-0310. PubMed PMID: 25153395.
2. Butt N, Bano F, Ghani M, Ahmed AM, Majeed N. Association of serum advanced glycation (AGEs) end products, apolipoprotein-B and zinc in severity of T2DM retinopathy.PJPS. 2021;34(2):803-8.
3. Majeed N, Ahmad TM, Hayat A, Israr S, Saddique A, Shabbir S. Role of Serum Lipid and Apolipoprotein Ratios in Prediction of Diabetic Retinopathy. Biosight. 2023;4(1):3-10. doi: https://doi.org/10.46568/bios. v4i1.85.
4. Lenters-Westra E, English. Evaluating new HbA1c methods for adoption by the IFCC and NGSP reference networks using international quality targets. CCLM. 2017;55(9):1426-34.
5. Gillery P. HbA1c and biomarkers of diabetes mellitus in Clinical Chemistry and Laboratory Medicine: ten years after. CCLM. 2023;61(5):861-72.
6. Rohlfing C, Hanson S, Weykamp C, Siebelder C, Higgins T, Molinaro R, et al. Effects of hemoglobin C, D, E and S traits on measurements of hemoglobin A1c by twelve methods. 2016;455(1):80-3.
7. Association AD. Addendum. 2 Classification and diagnosis of diabetes: standards of medical care in diabetes—2021. Diabetes care. 2021; 44(Supplement_1): S15-S33.
8. Jaisson S, Desmons A, Renard B, Chevelle B, Leroy N, Gillery PJCCA. Analytical performances of a new enzymatic assay for hemoglobin A1c. 2014; 434 (1): 48-52.
9. Berman M, Templin L, editors. Performance of the Enzymatic Alinity c Hemoglobin A1c Assay. 71st AACC Annual Scientific Meeting Abstracts; 2019: AACC.
10. Chakraborty S, Chakraborty S, Ghosh A, Chakraborty SS. A Comparative Evaluation of Estimation of Glycated Haemoglobin by Immunoturbidimetry and High Performance Liquid Chromatography in a Tertiary Care Hospital of Kolkata. IJRR. 2019;6(3):155-158.
11. Weykamp C, John G, Gillery P, English E, Ji L, Lenters-Westra E, et al. Investigation of 2 models to set and evaluate quality targets for HbA1c: biological variation and sigma-metrics. Clinical chemistry. 2015; 61(5): 752-9.
12. Stokes C, Frost S, Frost L, Mcinerney N, O'Dwyer S. A-070 A fully automated HbA1c Assay on the DxC 500

AU Clinical Chemistry Analyser. Clinical Chemistry. 2023;69(Supplement_1):hvad097. 67.

13. Clinical LSI. EP09-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 2013.
14. Petrides AK, Dahlin JL, Melanson SE. Method validation. Contemporary Practice in Clinical Chemistry: Elsevier; 2020. p. 57-75.
15. Cihan M, Abdullah Ü, KILINÇKAYA MF, Noyan T. Comparison of high performance liquid chromatography and turbidimetric inhibition immunoassay methods for measurement of hemoglobin A1c. JHSM. 2022; 5(5): 1281-5.
16. Lee JH, Kim S, Jun S-H, Seo JD, Nam Y, Song SH, et al. Analytical performance evaluation of the Norudia HbA1c assay. J. Clin. Lab. Anal . 2020; 34(11): e23504. doi: https://doi.org/10.1002/jcla.23504.
17. Núñez-Jurado D, Montenegro-Martínez J, Rubio-Sánchez R, Conde-Sánchez M, Domínguez-Pascual I. Evaluation of the Premier Hb9210 instrument for HbA1c determination. Scandinavian. J. Clin. Lab. 2023; 83(8):569-75.
18. Ohde S, Deshpande GA, Yokomichi H, Takahashi O, Fukui T, Yamagata Z. HbA1c monitoring interval in patients on treatment for stable type 2 diabetes. A ten-year retrospective, open cohort study. Diabetes research clinical practice. 2018;135(1):166-71.
19. Pasqualetti S, Carnevale A, Dolci A, Panteghini M. A step towards optimal efficiency of HbA1c measurement as a first-line laboratory test: the TOP-HOLE (Towards OPtimal glycoHemOgLobin tEsting) project. CCLM. 2022;60(3):441-50.
20. Braga F, Panteghini M. Verification of in vitro medical diagnostics (IVD) metrological traceability: responsibilities and strategies. Clinica Chimica Acta. 2014; 432(1):55-61.
21. Chaila MZ, Viniegra M, Gagliardino JJ, Martínez A, Simesen de Bielke MG, Frusti M, et al. Glycated Hemoglobin Measurement: Comparison of Three Methods Versus High Performance Liquid Chromatography. JDST . 2022;16(3):724-31. doi: 10.1177/ 193229682 1997179. PubMed PMID: 33686874.
22. Luis Monaco B, Pablo Salgado B, Carlos Buso M, Claudio Daniel Gonzalez M. Glycated Hemoglobin Measurement: Comparison of Three Methods Versus High Performance Liquid Chromatography. JDST. 2021;77(1):1-8.
23. Sriwimol W, Choosongsang P, Choosongsang P, Treerut P, Muenniam B, Makkong P, Pocathikorn A. Strong correlation and high comparability of capillary electrophoresis and three different methods for HbA1c measurement in a population without hemoglobinopathy. Scandinavian. J. Clin. Lab. 2020;80(2):139-50. doi: 10. 1080/ 00365513.2019.1703213.
24. Mukherjee B, Hooda C, Singh V, Sahu R, Singh MU. Comparison of NGSP Certified Methods and their Cost Analysis for Estimation of HbA1c-A Cross-sectional Study. JCDR . 2023;17(2):BC01-BC4.