AJ ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Analytical performances description of the immunoturbidimetric method for the determination of HbA1c using Selectra Pro M automated system at the Institut National d'Hygiène (INH) of Lomé

Gnatoulma KATAWA[1]*, Kossi ADJETE[1], Oukoe Marthe AMESSOUDJI[1], Wemboo HALATOKO[2,] Pélagie Edlom TCHADIE[1], Fagdeba BARA[1], Irène DOSSOU[1], Christèle NGUEPOU TCHOPBA[1], Damintoti Simplice KAROU[1] and Yovi AMEYAPOH[1]

[1]Unité de Recherche en Immunologie et Immunomodulation (UR2IM)/Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires (LAMICODA)/Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA), Université de Lomé, Lomé, Togo.
[2]Institut National d'Hygiène (INH), Lomé, Togo.

HbA1c is the best indicator for monitoring glycemic control in both type 1 and type 2 diabetic patients. Its value allows us to predict the development of long-term degenerative complications of diabetes. Several techniques offered by different laboratories allow the determination of HbA1c based on different principles. The purpose of the present study was to perform the verification of the analytical performances of the Selectra Pro M automated system, using immunoturbidimetry as the HbA1c assay method. Risk assessment of the method was performed; the analytical performances of the assay process was evaluated and the immunoturbidimetric method on the Selectra Pro M was compared to the capillary method on the Capillarys 2 Flex Piercing. Reproducibility and intermediate precision were satisfactory with CV in the range of 1.78 to 1.96% for the low level of Internal Quality Control (IQC) (HbA1c = 5.4%) and 0.82 to 2.28% for the high level of IQC (HbA1c = 11.3%). The reagent was linear from 2.5 to 16% (4 to 151 mmol/mol). The accuracy was considered satisfactory. The linear regression calculation (least squares line) showed an excellent correlation (R^2= 0.96) between the two techniques with an equation of the type [Capillarys] = 1.13 [Selectra Pro M] - 0.83. All documented and tested performances correspond to the performance required for HbA1c assay at INH of Lomé.

Key words: HbA1c, diabetes, performances of method, immunoturbidimetry, INH-Togo.

INTRODUCTION

Glycated hemoglobin (HbA1c) represents 4 to 6% of total hemoglobin. It results from the condensation of a glucose molecule with the N-terminal valine group of each of the two beta chains of hemoglobin A (Schnek and Schroeder, 1961). HbA1c represents a powerful tool for monitoring long-term glycemic control (Goldstein and Little, 1997; Vassault et al., 2010). In diabetics, a high concentration of HbA1c is associated with a wide variety of complications.

Its dosage is therefore essential to evaluate the management of diabetic subjects and the dosage technique used must be high-performance. The interest generated by this determination is at the basis of the diversity of its assay techniques having different reference value. This variability between different techniques means that the performance of the technique to be used must be verified before it is adopted. The analytical performances verification of a technique consists of evaluating the performance of the analytical process, quantifying it by following a standardized operating protocol and then evaluating it against defined criteria (COFRAC, 2015). In order to meet the requirements of its customers and bring out reliable results, the Institut National d'Hygiène (INH) of Lomé started implementing in 2002 a Quality Control process with the major commitment to be part of a logic of continuous improvement of its services. Since March 2011, special focus is placed on the Medical Biology Laboratories (MBL) for the accreditation according to ISO 15189 standard. All critical equipment newly acquired at the biochemistry laboratory of the INH is submitted to an on-site verification of all parameters available according to ISO 15189. However, some parameters including HbA1c have not yet gone through this process. In order to contribute to the improvement of the analytical performances, we initiated this study with the overall objective of verifying the analytical performances of the newly installed Selectra Pro M automated system that works on the immunoturbidimetric principle to measure HbA1c. The risks involved in applying the HbA1c assay method were assessed; then, the analytical performances of the assay process was evaluated; and finally, a comparison of the immunoturbidimetric method on Selectra Pro M to the capillary method on Capillarys 2 Flex Piercing was done.

METHODOLOGY

Assay techniques

Samples

Sixty-four whole blood samples collected on EDTA tubes (5 ml) were randomly selected. Samples were from individuals having hemoglobin A on the chromatographic profile.

Principles of measurements

Immunoturbidimetry on Selectra Pro M (ELI Tech Group, Puteaux, France): On Selectra Pro M, HbA1c assay technique is based on the principle of immunoturbidimetry and the HbA1c level is

calculated from a non-linear calibration curve obtained from four standards of different levels and a zero point. Two control levels, low and high (ELI Tech Control L+H), are assayed to each series.

Capillary method on Capillarys 2 Flex Piercing (SEBIA, Lisses, France): On Capillarys 2 Flex Piercing, HbA1c measurement is based on the principle of capillary electrophoresis in free solution. It allows the separation of charged molecules according to their own electrophoretic mobility in a given pH buffer, and according to the pH of the electrolyte, from a more or less important electro-osmotic flow. The separation is achieved by applying a potential difference of several thousand volts at the terminals of each capillary.

Technical steps of the verification protocol

The evaluation was performed in 3 time points:

(1) One week of familiarization and learning about the device;
(2) Two weeks during which repeatability tests were performed at 2 concentration levels (low, high); and reproducibility tests calculated from the results of samples tested in 2 different series per day for 10 days;
(3) Three weeks during which the comparison between the HbA1c values of the different machines was performed.

Risk assessment

The 5M method was used by considering all the critical points (strengths and weaknesses) concerning: (1) the premises and environmental conditions (layout, temperature); (2) reagents (preparation, batch-to-batch variations and stability); (3) equipment (compliance with supplier operating procedures and instructions, maintenance, calibration, metrological connection); (4) staff (training, evaluation of skills); (5) method (performance criteria: precision, accuracy, uncertainties, interferences), taking into account the quality criteria of the samples analyzed.

Evaluation of the performance of the assay method

The analytical evaluation protocol was inspired by COFRAC's SH-GTA 04 (4) reference protocol.

Repeatability assessment: For the repeatability assessment, 20 assays were performed in the same series, on the same day, with the same procedure, the same operator, the same batch of reagent and the same working conditions, with two levels of control. The coefficient of variation (CV) was used to evaluate the repeatability of the method expressed as a percentage. CV was calculated using this formula:

$$m = \frac{\sum x_i}{n} \qquad S = \sqrt{\frac{\sum (x_i - m)^2}{n-1}} \qquad CV \text{ en } \% = \frac{(S) \times 100}{m}$$

where m is the mean value of Hb1Ac measured, n is the number of

*Corresponding author. E-mail: mahkatawa@yahoo.fr.

tests, S is the standard deviation.

This calculated CV was compared to the admissible limit CV given by suppliers or learned societies such as Société Française de Biologie Clinique (SFBC).

Assessment of the intermediate precision: The intermediate precision was determined from the results obtained on control specimens at two concentration levels, assayed daily in 2 different series per day for 10 days, by varying the operating conditions (operator, calibration, batches of reagents, etc). The calculation methods were similar to those for repeatability. The CV calculated on the experimental values of each series is compared to the admissible limit CV.

Accuracy approach: The accuracy, quantified by bias, was estimated by comparing the mean (m) to the expected target value assimilated to the true value (v). The m value is obtained during the intermediate precision study (intra-laboratory reproducibility) and established with samples of internal quality control. The bias is expressed as a percentage of the target value.

$$\text{Bias } (\%) = 100 \, x \, \frac{(m-v)}{v}$$

Comparison of methods: The accuracy of the immunoturbidimetric technique was assessed against the Capillary technique. To do this, 64 individuals' whole samples were analyzed on the two devices in a short time. The comparison between the two methods was made using 4 tools:

(1) The graph of ratios of the values from Selectra to those from Capillarys;
(2) The equation of the regression line according to the method of least squares and the determination of the regression coefficient;
(3) The analysis of the diagram of the differences between the 2 techniques according to the instructions of Bland and Altman.
(4) The t test of the differences to see if the differences observed between the results are statistically significant. The calculated t was compared to the theoretical t with (n-1) DOF (n is the number of tests and DOF the degree of freedom).

Statistical analysis

The repeatability, reproducibility and bias data were entered and analyzed in Microsoft® Excel 2010 spreadsheet. The comparison of the methods was made with the R 3.3.1 software. The differences were considered significant at a value of P less than 0.05 (P<0.05).

RESULTS AND DISCUSSION

Risk assessment

The results of the risk assessment using the 5M method showed ten strong points and seven weak points which are presented in Table 1.

Performance evaluation

Repeatability of Selectra Pro M

The CV from the repeatability calculation for low and high

IQC was 1.78 and 0.82, respectively (Table 2). These obtained CV are lower than the supplier and SFBC CV. We then deduced that our repeatability data are consistent.

Intermediate precision

The low and high IQC CV are 1.96 and 2.28, respectively. These CV are lower than the supplier's and SFBC's CV. It can then be inferred that the intermediate precision data on the Selectra Pro M are compliant (Table 3).

Approach to accuracy

The biases of the low and high IQC are 0.92 and 0.044, respectively, and are lower than the SFBC accuracy values (Table 4).

Comparison of Selectra Pro M and Capillarys methods

The reports graph: The graphical representation of the Selectra/Capillarys ratios (Figure 1) shows a homogeneous distribution of results over all the HbA1c values studied and are in the range [0.8; 1.2], therefore close to 1.

Regression line or Passing-Bablock for Selectra Pro M and Capillarys methods: The regression line shows a good correlation between the two methods (Figure 2).

Bland-Altman graph: Good agreement was obtained for HbA1C values below 16% (Figure 3). The mean difference md= -0.19%. Most of the points are in the range [-1.41; 1.02], the range of agreement limits (md ± 2sdd). Thus, of the 64 values compared, only 3 are outside the approval limits.

The difference t-test

The t-test of differences was performed on the two sets of results given by the two devices. The mean of the differences (md) was calculated. The calculated t was compared to the theoretical t with (n-1) DOF. Table 5 shows the data of the t-test of differences. The results of the two analytical systems therefore showed statistically significant differences at the 5% risk threshold (Table 5).

DISCUSSION

This study was initiated in order to verify the analytical performances of a newly installed Selectra Pro M automated system that uses the immunoturbidimetric

Table 1. Risk assessment with the 5M method.

5M	Critical points	Strong points	Weak points
Matter (reagents)	Storage and conditions of use Reagents and standards reconstitution	Good reagents storage measures Compliance with the operating procedure and reagent supplier manual	Failure to calibrate the micropipettes periodically Lack of metrological traceability
Medium	Local Environmental conditions	Good metrology and monitoring of enclosures Static environmental conditions over time	Recording of environmental conditions not updated
Material	Drift monitoring Contamination	Compliance with supplier's operating procedures and instructions. Periodic maintenance, calibration	Operating procedure not written for HbA1c
Method	Performance criteria Cause of measurement uncertainty	On-site verification for some parameters ; Compliance with non conformity management procedures	No on-site verification for HbA_{1c} Lack of calculation of measurement uncertainties of quantitative parameters
Manpower (Staff)	Skills and skill maintanance	Training planning and staff assessment Authorized personnel for HbA1c dosing.	Lack of a personnel evaluation grid for HbA1c testing

Table 2. Repeatability study of Selectra Pro M.

Sample (IQC)	Number of values (n)	Mean (m)	Standard deviation	CV (%)	CV (%) supplier	CV (%) (SFBC)	Conclusion
IQC Low (5.4)	20	5.235	0.093	1.78	2	3.8	Compliant
IQC High (11.3)	20	11.335	0.093	0.82	3	3.8	Compliant

*Société Française de Biologie Clinique (SFBC).

Table 3. Intermediate Reliability Study of Selectra Pro M.

Sample	Number of values (n)	Mean (m)	Standard deviation	CV (%)	CV (%) supplier	CV (%) (SFBC)*	Conclusion
IQC Low (5.4)	20	5.35	0.105	1.96	2	5	Compliant
IQC High (11.3)	20	11.31	0.258	2.28	3	5	Compliant

*Société Française de Biologie Clinique (SFBC).

method to measure HbA1c. Indeed, a "scope A verification" was already carried out where the recognized methods (CEmarked IVDDs or "supplier" methods) are validated in their field of application. Our analytical performances verification of the HbA1c assay method compared

Table 4. Accuracy approach of Selectra pro M.

Sample	Results (%)	Ref Val. v (%)	Difference (%)	Bias (%)	Bias (SFBC) (%)	Conclusion
IQC Low	5.35	5.4	0.05	0.92	6.2	Compliant
IQC High	11.305	11.3	0.005	0.044	6.2	Compliant
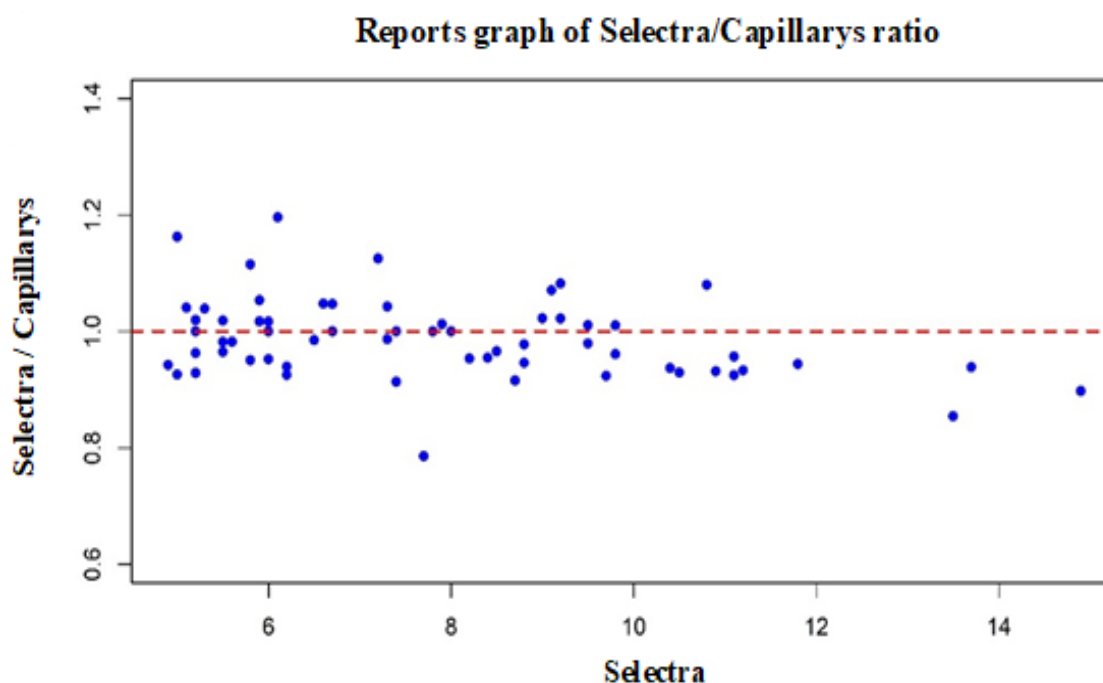
Figure 1. Representation of the Selectra Pro M/Capillarys ratio graph. The points on the graph represent the ratio values between Selectra Pro M and Capillarys. All values are between 0.8 and 1.2.

the results from Selectra Pro M to those of the mirrored Capillarys 2 Flex Piercing.

The evaluation of the risks according to the different critical points, in spite of the different strong points, still showed weak points to be improved. In particular, calibration of micropipettes and metrological traceability to ensure proper packaging and reconstitution of reagents; recording and updating environmental conditions to avoid their influence on the technique; writing the handling procedure to ensure the suitability of the material to be used; on-site verification and calculation of uncertainties to evaluate the performance of the method and having a personnel evaluation grid to ensure the qualification of the personnel.

After risks assessment, we evaluated the performance of this method by evaluating the repeatability, the intermediate precision, and accuracy assays.

The CVs for the repeatability study complied and generally met the requirements issued by the supplier and also the criteria of the VALTEC protocol (SFBC). Similarly,

the CVs achieved were similar to those of Beaune et al. (2009) with CV ranging from 1.18 to 1.91% in the evaluation of a technique for the determination of HbA1c on Architect CI8200 using an immunoturbidimetric technique. However, Samaan et al. (2007) and Urrechaga, (2018) found CVs of less than 1% whatever the level measured and whatever the material used (fresh blood or lyophilized control), CVs of 0.42 to 0.30% and 0.71 to 0.43%, respectively. This difference can be explained by the technique used (HPLC), which is a much more precise technique.

The CVs for the intermediate precision study showed reproducibility in accordance with the supplier's requirements. These CVs are consistent with those of Beaune et al. (2009) and El Arabi et al. (2013) in the evaluation of DCA Vantage which used an immunological agglutination technique for the determination of HbA1c and found CVs ranging from 2.09 to 2.64% and 0.9%, respectively, while Urrechaga, (2018) found more satisfactory CVs of 0 to 0.36%.

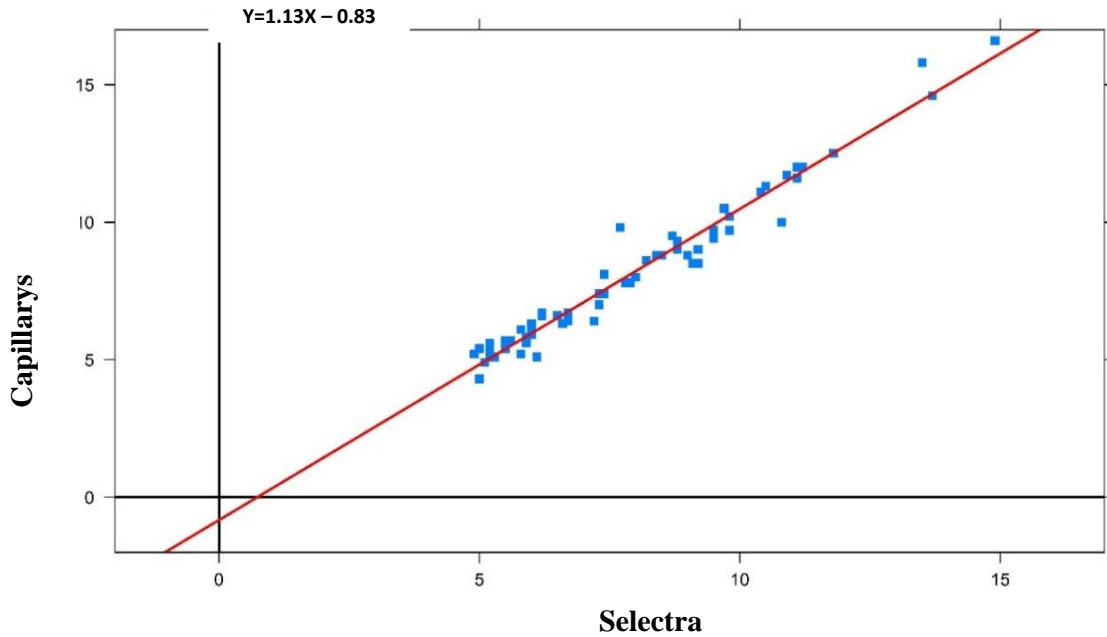## Least squares line, orthogonal regression

Figure 2. Regression between Selectra and Capillarys values. The right side of the graph represents the correlation of the intercept capillary technique on the Capillarys versus the immunoturbidimetric technique on Selectra Pro M. Intercept at origin (Intercept) = -0.83, Regression coefficient (R^2) = 0.96.
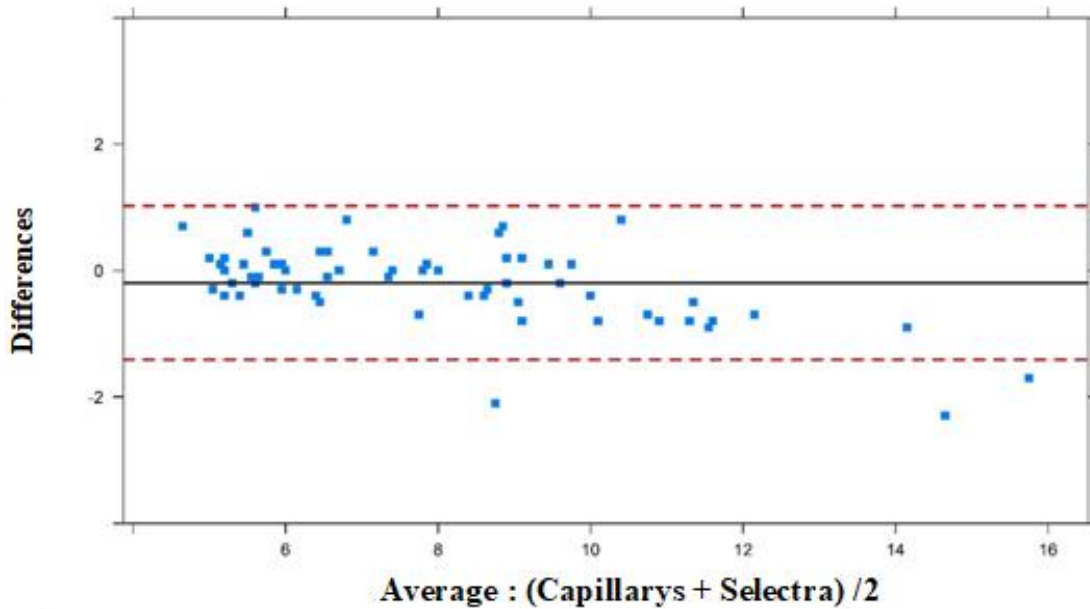
Bland-Altman graph

Figure 3. Representation of the Bland-Altman graph of Selectra Pro M and Capillarys methods. The points on the graph represent the average of the differences in the values of the two methods. Only 3 points are outside the approval limits.

Table 5. Data from the t-test of differences between Selectra Pro M and Capillarys.

Variable	Analytical system		Difference
	Selectra Pro M	Capillarys	Selectra-Capillarys
Average (m_d)	7.82	8.00	-0.19
t calculated	2.55		
DOF	63		
p (value)	0.01		

During a method evaluation, the measurements may be close to each other (good precision) but outside the probability range of the true value, they are therefore biased (bad precision) or vice versa. However, a method is said to be accurate if it is both faithful and fair. In view of the level of biases obtained in this study and thus compared with the SFBC specifications, the immunoturbidimetric method is considered accurate.

The relevant data brought by the equation of the regression line whose slope (1.13) and intercept at the origin (-0.83) express the similarity of the methods compared. Beaune et al. (2009) correlated Architect Abbott (Immunoturbidimetry) with HPLC D-10 Bio-Rad and found comparable results (R^2 = 0.98 for 161 samples tested) with a regression line of equation [Abbott] = 1.02 [Bio-Rad] -0.636. Grant et al. (2017) and Berlanda et al. (2020) found also the same R^2=0.96 in respectively making comparison between D10 and Quo-Test with an equation of the type: Quo Test = 0.94 [D10] +4.93 and the evaluation of an automated immunoturbidimetric assay for detecting canine C-reactive protein.

The mean difference (md= -0.19%) indicates that the results obtained with the Selectra Pro M are slightly lower. Several studies have reported interferences between the other variants of hemoglobin when measuring the HbA1c (Little et al., 2008; Little and Roberts, 2009; Lee et al., 2011). This decrease of up to 1.41% (-2sd) in the results is explained by the fact that an underestimation of HbA1c was reported with immunological tests in the presence of high fetal hemoglobin concentrations as stated (Adekanmbi et al., 2016). The t-test of difference questions the transferability between the two (2) methods. It is commonly accepted in the diabetes community that a 0.5% difference in HbA1c empirically reflects a 1 mmol/L (approximately 0.25 g/L) change in mean blood glucose levels over the last 120 days prior to sampling (Simmons and Hlaing, 2014). Such variations for HbA1c values of less than 8% may lead to erroneous changes in the treatment of the diabetic (1). Intensification of therapy may be associated with side effects such as hypoglycemia with all its consequences (Seaquist et al., 2013) and a falsely assumed positive trend in HbA1c could be detrimental to the patient through continued poor metabolic control (Roth et al., 2018).

Conclusion

The assessment and control of risks in the context of this on-site verification enabled the implementation of the necessary actions to reduce and/or eliminate the potential risks identified. The performance criteria evaluated (repeatability, reproducibility and the approach to accuracy) are in accordance with the requirements of the supplier and the SFBC learned society and therefore reliable and fair. When comparing the two methods, despite the good correlation between them, there are still statistically significant differences at the 5% risk level. Therefore, even though both techniques have good accuracy, it is important to always follow the patients with the same technique or to be aware of the differences. All performances documented and tested meet the performance requirement of HbA1c assay. The immunoturbidimetric method is therefore declared suitable for the HbA1c assay.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

REFERENCES

Adekanmbi J, Higgins T, Rodriguez-Capote K, Thomas D, Winterstein J, Dixon T (2016). Erroneous HbA1c results in a patient with elevated HbC and HbF. Journal of Clinica Chimica Acta 462:153-7.

Beaune G, Ducruet J, Jund J, Favre S (2009). Evaluation of a technique for determining HBA 1C on Architect CI8200 (Abbott Diagnostic): Comparison with Bio-Rad HPLC D-10 technique. Annales de Biologie Clinique 67:1

Berlanda M, Valente C, Bonsembiante F, Badon T, Bedin S, Contiero B (2020). Evaluation of an automated immunoturbidimetric assay for detecting canine C-reactive protein. Journal of Veterinary Diagnostic Investigation 32(6):948-52.

COFRAC (2015). Technical guide to accreditation, verification (scope A) /

validation (scope B) of medical biology methods (SH GTA 04) Revsion 01:191p.

El Arabi H, Willems D, Melot C, Dorchy H (2013). Evaluation of the DCA Vantage for rapid in-office HbA1c measurement on capillary blood in young type 1 diabetic patients. Revue Médicale de Bruxelles 34(2):87-89.

Goldstein D, Little R (1997). Monitoring glycemia in diabetes: short-term assessment. Journal of Endocrinology and Metabolism Clinics of North America 26(3):475-486.

Grant D, Dunseath G, Churm R, Luzio S (2017). Comparison of a point-of-care analyser for the determination of HbA1c with HPLC method. Journal of Practical Laboratory Medicine 8:26-29.

Lee SC, Wang LH, Tsai SM, Fang HY, Tsai LY (2011). Effects of the Hb E, Hb H and Hb G-Taichung variants on HbA1c values by the Bio-Rad variant™ II turbo analyzer. Journal of Clinical biochemistry 44(16):1338-1342.

Little RR, Roberts WL (2009). A review of variant hemoglobins interfering with hemoglobin A1c measurement. Journal of Diabetes Science Technology 3(3):446-451.

Little RR, Rohlfing CL, Hanson S, Connolly S, Higgins T, Weykamp CW (2008). Effects of hemoglobin (Hb) E and HbD traits on measurements of glycated Hb (HbA1c) by 23 methods. Journal of Clinical chemistry 54(8):1277-1282.

Roth J, Müller N, Lehmann T, Böer K, Löbel S, Pum J (2018). Comparison of HbA1c Measurements using 3 Methods in 75 Patients Referred to One Outpatient Department. Journal of German Society of Endocrinology and Diabetes Association 126(1):23-26.

Samaan S, Lesellin A, Baron S, Fonfrede M (2007). Evaluation of the G8 analyzer and PIANO software (Tosoh Bioscience) for hemoglobin A1c determination. Journal of Spectra Biologie 161:38-45.

Schnek A, Schroeder W (1961). The relation between the minor components of whole normal human adult hemoglobin as isolated by chromatography and starch block electrophoresis. Journal of the American Chemical Society 83(6):1472-1478.

Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L (2013). Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. The Journal of Clinical Endocrinology Metabolism 98(5):1845-1859.

Simmons D, Hlaing T (2014). Interpretation of HbA1c: association with mean cell volume and haemoglobin concentration. Journal of Diabetic Medicine 31(11):1387-92.

Urrechaga E (2018). Analytical evaluation of the ADAMS™ A1c HA 8180T analyzer for the measurement of HbA1c. Journal of Clinical Laboratory Analysis 32(1):e22155.

Vassault A, Hulin A, Chapuzet E, Arnaud J, Giroud C (2010). Verification/validation of analytical method performance. Annales de Biologie Clinique.