Practical Laboratory Medicine 31 (2022) e00288

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

External quality assessment scheme for HbA1c assays in Thailand: A 5-year experience

Supaporn Suparak [a,*], Busadee Pratumvinit [b], Kanokwan Ngueanchanthong [a], Petai Unpol [a], Ariya Thanomsakyuth [c], Chavachol Setthaudom [c], Mongkol Kunakorn [c], Archawin Rojanawiwat [a], Ballang Uppapong [a]

[a] Department of Medical Sciences, Ministry of Public Health, Thailand
[b] Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
[c] Faculty of Medicine Ramathibodi Hospital, Thailand

ARTICLE INFO

Keywords:
Accuracy-based
Diabetes mellitus
External quality assessment
HbA1c
Proficiency testing

ABSTRACT

Background: Thailand National External Quality Assessment Scheme (NEQAS) for HbA1c was established to evaluate the quality of HbA1c assays in Thailand in 2016.
Methods: HbA1c results from participating laboratories were compared to the target value assigned by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference system.
Results: The pass rates of participating laboratories during 2016–2020 were72–88%. The mean bias ranged between −0.19 and 0.20% of HbA1c. SD ranged from 0.30 to 1.08% of HbA1c. The overall coefficients of variation ranged from 4.46-15.66%.
Conclusions: Performance evaluation using IFCC assigned values indicated that different assay methods had an effect on HbA1c results. Participation in external quality assessment programs for HbA1c analysis is essential for improving laboratory quality and benefiting patient management.

1. Introduction

The precision and accuracy of hemoglobin A1c (HbA1c) measurements are critical for monitoring and diagnosing diabetes mellitus [1,2]; therefore, clinical laboratories must constantly monitor the performance of their assays. External quality assessment (EQA) programs are a tool for laboratories to verify and evaluate the performance of their HbA1c assays. EQA is an interlaboratory comparison program in which EQA providers send sample panels to participating laboratories for analysis on a regular basis. Individual laboratories compare their results to those of other laboratories in a peer group or to an assigned value [3]. Comparing results with the assigned value is better at reflecting the accuracy of HbA1c testing, which is necessary for clinical diagnosis and monitoring using the HbA1c. This article reveals the experience of setting up an accuracy-based EQA scheme with the help of the European Reference Laboratory for Glycohemoglobin in values assignment with the International Federation of Clinical Chemistry (IFCC) secondary reference measurement procedures.

Currently, HbA1c laboratory-based assays are commonly based on five principles: liquid chromatography [high-performance liquid chromatography (HPLC), capillary electrophoresis, affinity binding chromatography, immunoassay, and enzymatic methods [2].

* Corresponding author.
E-mail address: supaporn.su@dmsc.mail.go.th (S. Suparak).

https://doi.org/10.1016/j.plabm.2022.e00288
Received 24 January 2022; Received in revised form 26 April 2022; Accepted 20 June 2022
Available online 27 June 2022

Apart from laboratory-based HbA1c assays, the point-of-care (POC) analyzers were also often used in community setting, point-of-care HbA1c brought evidence-based primary care to villages [4]. However, the performance of POC HbA1c testing devices varies significantly across individual studies, limiting their application for diabetes screening and diagnosis [5]. Each method has limitations in its application, particularly in the presence of interferences; therefore, the HbA1c assay must be standardized to reduce variations between results obtained by various methods.

The IFCC and National Glycohemoglobin Standardization Program (NGSP) [6] cooperatively have successfully achieved the HbA1c standardization, significantly reducing differences between results obtained by various commercial methods. In addition to using NGSP and IFCC certified methods to achieve a precise and accurate HbA1c measurement, the assays' performance with EQA materials must be monitored periodically. Previously, because of the unavailable targeted EQA scheme, all HbA1c EQA programs in Thailand were the peer group comparison. However, the National EQA HbA1c program was established in Thailand for the first time in 2016 to assess HbA1c based on target values assigned by the European Reference Laboratory for Glycohemoglobin (ERL) by using 4 IFCC calibrated secondary reference measurement procedures. This ISO/IEC 17043: 2010 certified accuracy-based HbA1c EQA program provides high-quality, affordable materials for clinical laboratories throughout the country [7]. In addition, the ISO 13528:2015 [8] quality standard system was used in the EQA program's statistical method.

2. Materials and methods

2.1. Sample preparation and value assignment

EQA samples were prepared in Ramathibodi Hospital using pooled human ethylene diaminetetraacetic acid residual of patient whole blood samples. The samples were tested for HbA1c values using the turbidimetric inhibition immunoassay method (Cobas c501, Roche Diagnostics, Mannheim, Germany) and HbA1c values were verified using turbidimetric inhibition immunoassay method (Cobas c513) at the Faculty of Medicine Siriraj Hospital. Both the Ramathibodi and Siriraj Hospital laboratories are NGSP level I certified which received yearly certification of traceability to the Diabetes Control and Complications Trial and a quarterly monitoring [9].

The samples were screened for hepatitis B, hepatitis C and HIV-1 viruses by chemiluminescence immunoassay (Architect, Abbott Diagnostics, Abbott Park, IL).

The aliquot samples were sent to the ERL in the Netherlands in two shipments per year (2016–2017) and in one shipment per year (2018–2020) to obtain the HbA1c values with four secondary reference methods in duplicate, and the mean was calculated for the assigned value. Secondary reference methods included Roche TQ generation three on Cobas c513 (immunoassay), Tosoh G8 (ion-exchange), Trinity Biotech Premier Hb9210 (affinity chromatography), and Abbott Alinity (enzymatic assay). The samples were aliquoted into 500 ml per tube and stored at −70 ∘C until they were delivered to participating laboratories. This study was approved by the local Institutional Review Board (Ref: MURA2016/27), which waived the requirement for informed consent.

2.2. Sample packaging and delivery to participating laboratories

Frozen EQA samples are shipped twice a year (2016–2017) and three times a year (2018–2020), with four to five sample panels in each cycle. The transportation company transported the EQA samples on dry ice and delivered them directly to the laboratory (door to door) within 24 h. During the transportation, a temperature monitoring system was installed. The participating laboratories would be notified of the sample delivery date in advance. The participating laboratories were required to check the samples' condition as soon as they arrived. If the samples were not analyzed immediately, they were stored at 2–8 °C.

2.3. Statistical analysis accuracy assessment

The accuracy performance was evaluated by the percent difference of HbA1c values the participating laboratories and the IFCC-assigned value as follows:

$$\% \text{ Difference} = (\text{Xi} - \text{IFCC assigned value}/\text{ IFCC assigned value}) \times 100$$

where Xi = % HbA1c value from a participating laboratory.

Acceptable limits were within ±10% (year 2016–2017), ±9% (year 2018–2019), and ±8% (year 2020).

The difference of mean HbA1c values between each assay method and the assigned value was compared using paired *t*-test analysis by using SPSS 26.0 statistical software package (SPSS, inc., Chicago, IL, USA). The $P < 0.05$ was considered statistically significant.

2.4. Precision assessment

The precision was assessed using duplicated test samples and statistical analysis for within and between-laboratory Z-scores, as follows:

$$\text{Between laboratory Z-score} = \text{Standardized sum}_i\ (SS_i) - \text{Median}_{SS}/\ \text{NIQR}_{SS}$$

$$\text{Standardized difference}_i = (Sample\ 1 - Sample\ 2)\%\sqrt{2}$$

where $median_{SS}$ is median of standardized sum, and $NIQR_{SS}$ is normalized interquartile range of standardized sum, respectively.

Within laboratory Z-score = Standardized $difference_i$ (SD_i)-$Median_{SD}/NIQR_{SD}$

$$\text{Standardized difference}_i = (Sample\ 1 - Sample\ 2)\%\sqrt{2}$$

where $median_{SD}$ is median of standardized difference, and $NIQR_{SD}$ is normalized interquartile range of standardized difference, respectively.

The acceptable criteria was $|\text{Z-score}| < 3.00$.

The mean, mean bias, SD, CV, and relative bias of each peer group as well as total results were calculated as follows:

Mean %HbA1c = ΣXi / number of participating laboratories

Mean Bias = Mean% HbA1c - assigned value

$$SD = \sqrt{\frac{\sum (X_i - \text{Mean \% HbA1c})^2}{\text{no. lab} - 1}}$$

Where X_i = % HbA1c value from participating laboratories

% coefficient of variation (%CV) = (SD × 100) / Mean% HbA1c

% Relative bias = (Mean % HbA1c – assigned value) x 100 / assigned value

2.5. Homogeneity test

Ten EQA samples were randomly selected from each set and analyzedfor within-sample variation by Cochran's range test. If Cochran expected value (C_{exp}) < Cochran critical value (C_{crit}), there was no significant difference in each tube.

$C_{exp} = D^2_{max} / \sum D_i^2$

Where D^2= (Dup1 - Dup2)^2

D^2 max = D^2 maximum value

According to ISO13528:2015, the sample homogeneity was assessed by comparing the between-sample standard deviation (S_S) with the maximum permissible error criterion for differences (δ_E). The proficiency test items are considered adequately homogeneous if $Ss \leq 0.1\delta E$.

2.6. Stability test

The isochronous stability of the EQA samples panel was performed. The mean %HbA1c of the samples stored at −70 ∘C ($\bar{y}_1$) was compared with sample stored at 2–8∘C for two weeks ($\bar{y}_2$). The samples were considered to be adequately stableif $|\bar{y}_1 - \bar{y}_2| \leq 0.1\delta_E$ according to ISO13528:2015.

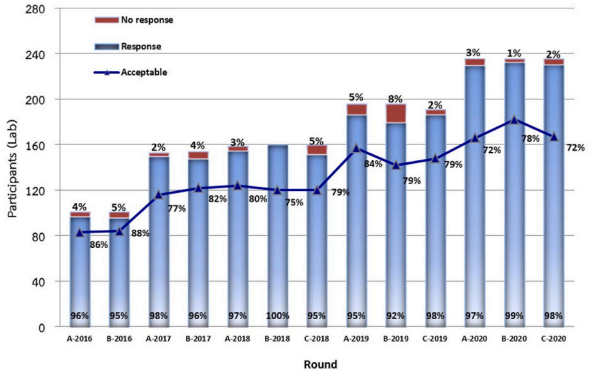

Fig. 1. The number of participating laboratories, response, and pass rates in each cycle during 2016–2020.

Table 1
Manufacturers and instruments used for HbA1c analysis during 2016–2020.

Manufacturer	Instruments	Number of participants report				
		2016 (20 Instruments)	2017 (27 Instruments)	2018 (34 Instruments)	2019 (40 Instruments)	2020 (53 Instruments)
Archem Diagnostic	Dirui CS 300B	–	2	5	4	3
Beckman Coulter, Inc	DxC-300	1	–	–	–	–
	Beckman Coulter LX20Pro	–	4	1	–	–
	Beckman Coulter AU400	–	–	3	6	4
	Beckman Coulter AU480	–	–	3	7	4
BioSystems	BioSystems BA400	–	–	–	–	2
Drawbridge Health	Olympus AU480	–	4	1	1	–
Furuno Electric Co. Ltd.	Furuno CA-800	–	–	–	–	2
Getein Biotech, Inc.	Getein 1600	–	–	–	–	2
Guangzhou Wondfo Biotech Co., Ltd.	Finecarewonfo	–	–	5	3	–
	Finecare™ FIA Meter	–	–	–	–	5
Home Access Health Corporation	Beckman Couter AU680	–	–	–	3	2
Ortho Clinical Diagnostics	Vitros 4600	–	–	2	5	6
	Vitros 5600	–	–	–	3	5
	Vitros 7600	–	–	–	–	1
Randox Laboratories Ltd	Rx Imola	2	2	6	12	12
	RX modena	–	–	–	–	2
	Cobas c502	1	2	–	2	6
	Cobas 8000	1	–	3	–	–
	Cobas c311	7	5	6	6	6
	Cobas c111	11	9	6	9	18
	Cobas Integra 800	17	4	2	–	–
	Cobas Integra 400 Plus	27	33	54	49	63
	Cobas c501/Cobas 6000	96	131	213	222	275
	Cobas c513	–	2	5	4	9
	Cobasc503	–	–	–	–	11
	Cobas Pro	–	–	–	–	1
Siemens Healthcare	Siemen Dimension EXL200	2	5	2	2	7
	Siemen Dimension RXL	–	2	4	5	2
	DCA Vantage Analyser[a]	–	–	–	–	1
Thermo Fisher Scientific Oy	Konelab prime 60	2	2	2	1	–
	Konelab 20i	–	–	–	1	–
Abbott Diagnostics	Architect C4000/ C8000/Ci4100	2	22	32	35	45
	Alinity ci-series	–	–	–	4	14
BIOZEN	XL-640/Cromatest	–	2	1	–	–
DiaSys Diagnostic Systems GmbH	Sysmex BX-3010/ Sysmex BX-4000	1	25	39	46	41
JEOL Ltd.	BIOMAJESTY JCABM6010/C	–	14	17	25	39
Shenzhen Mindray Bio-Medical Electronics Co., Ltd.	Mindray BS400	–	–	2	3	7
	Mindray BS240	–	–	–	1	2
	Mindray BS800	–	–	–	–	8
	Mindray BS360E	–	–	–	–	1
	Mindray BS430	–	–	–	–	1
	Mindray BS480	–	–	–	–	1
Siemens Healthcare Diagnostics Inc	Advia 1800	–	–	–	–	1
Arkray, Inc.	ADAMSTM A1C Lite HA-8380V	2	–	–	–	5
	ADAMS A1c HA-8160	2	–	–	–	–
	ADAMS A1C HA-8180	11	10	25	14	10
	ADAMS A1c HA-8180V	–	–	11	37	40
Bio-Rad Laboratories	BIORAD D10	2	2	–	6	4
Jiangsu Audicom Medical Technology Co., Ltd.	Audicom AC6600	–	–	–	–	2
	MQ-2000PT	–	–	–	–	6

(*continued on next page*)

Table 1 (*continued*)

Manufacturer	Instruments	Number of participants report				
		2016 (20 Instruments)	2017 (27 Instruments)	2018 (34 Instruments)	2019 (40 Instruments)	2020 (53 Instruments)
Shanghai Huizhong Medical Science and Technology Co. Ltd (China)						
Shenzhen Labnovation Technologies	LD500 HbA1c	–	1	2	–	–
	LABNOVATION	–	1	–	–	–
	GH series (GH900)	2	1	–	–	–
	Lifotronic H9	–	1	3	2	2
Tosoh Corporation	Tosoh HLC-723GX	–	–	5	8	7
	Tosoh HLC-723G8	–	–	–	–	1
Greencross Medical Science	Arkray Pocket Chem A1c Advanced[a]	–	2	–	–	–
EKF Diagnostics GmbH	Quo-Lab HbA1C[a]	–	–	2	1	–
	Quo-Test HbA1c[a]	–	–	3	2	1
Green Cross Medis Corp.	LabonaCheckA1C[a]	–	–	2	–	–
	CERA-STAT 4000[a]	–	–	–	2	2
OSANG Healthcare Co.,Ltd.	CLOVER A1c TM Self[a]	–	3	3	8	5
	HemoCue® HbA1c 501[a]	–	–	–	3	–
Trinity Biotech	Premier Hb9210	3	6	–	–	1
WuxiBiohermesBio&MedicalTechnology Co., Ltd.	A1c check pro[a]	–	–	–	2	9
Sebia	Capilarys 3 TERA	–	–	–	1	3

[a] Point of care testing.

3. Results

3.1. EQA sample panels

The EQA sample panels were hepatitis B, hepatitis C and HIV-1 viruses free. The percentage difference in HbA1c values was within acceptable limits across all three laboratories (Faculty of Medicine Ramathibodi Hospital, Faculty of Medicine Siriraj Hospital, and IFCC), indicating that there was no variation between them (data not shown). EQA sample panels were adequately homogeneous and stable at 2–8 °C for at least two weeks and at −70 °C for 1 year ($|\overline{y}_1 - \overline{y}_2| \leq 0.1\delta_E$).

3.2. Number of participating laboratories and response rate during 2016–2020

During the 2016–2020 period, the number of participating laboratories, including private and public members, increased from 101 to 236 laboratories. The response rates increased from 92% to 100% and the pass rates increased from 72% to 88% (Fig. 1).

3.3. HbA1c instrument used

The manufactures and instruments used for HbA1c analysis was shown in Table 1. The assays included liquid chromatography, capillary electrophoresis, affinity binding chromatography, immunoassay, and enzymatic methods. The methods were increased year after year from 20 in 2016 to53 in 2020. The majority of the assay methods used were immunoassays.

3.4. Laboratory performance

During 2016–2020, the pass rates by samples were 79.57–95.88%. The mean bias varied from −0.19- 0.20% of HbA1c, while the standard deviation (SD) were 0.30–1.08% of HbA1c. The overall coefficients of variation (%CV) ranged from 4.46 to 15.66% (Table 2).

3.5. The relative bias and variability classified by assay methods

Comparison with the assigned value by using paired *t*-test, the relative biases were −3.05 to +4.54% ($P = 0.248$) in immunoassay methods, −5.97 to +4.23% ($P = 0.745$) in enzymatic methods had, −11.13 to +1.90% ($P < 0.001$) in HPLC and −21.25 to +31.19% ($P = 0.025$) in boronate affinity chromatography. Method-specific, between-laboratory CV ranged from 2.59% to 36.64%.

The immunoassay method had a CV of 3.15–15.52%; enzymatic methods had 5.74–21.69%; HPLC had 2.59–12.42%, Low-pressure liquid chromatography (not analyze, N = 1), Boronate affinity chromatography had 3.75–36.64% (Table 3).

Analysis of the bias of HbA1c testing by assay methods in the samples was divided into 3 groups: 1. HbA1c <6.3%, 2. HbA1c 6.3–6.7% and 3. HbA1c > 6.70% was shown in Fig. 2A and Table 4. The performance of the individual methods was demonstrated in Fig. 3, and found some instrument was out of acceptable criteria within ±8%.

Table 2
Accuracy performance and overall variability of participants' during 2016–2020.

Round	Sample	Number of participant reported	%Acceptable	Assigned IFCC value	Mean %HbA1c	Mean bias	SD	% CV
A-2016	01	97	87.63	4.98	4.90	−0.08	0.50	10.27
	02	97	94.85	5.97	5.92	−0.05	0.57	9.62
	03	97	95.88	6.87	6.81	−0.06	0.30	4.46
	04	95	94.74	9.15	9.09	−0.06	0.49	5.39
B-2016	01	96	90.63	6.53	6.47	−0.06	0.46	7.07
	02	96	91.67	8.82	8.74	−0.08	0.59	6.77
	03	96	90.63	5.54	5.51	−0.03	0.55	9.90
	04	96	91.67	6.51	6.47	−0.04	0.39	5.99
A-2017	01	150	82.67	5.32	5.22	−0.10	0.46	8.79
	02	150	93.33	8.81	8.78	−0.03	0.49	5.58
	03	150	84.67	5.33	5.23	−0.10	0.45	8.64
	04	150	88.00	6.76	6.68	−0.08	0.65	9.77
B-2017	01	148	88.51	7.56	7.39	−0.17	0.59	7.93
	02	148	85.81	5.58	5.44	−0.14	0.67	12.28
	03	147	90.48	6.41	6.23	−0.18	0.66	10.63
	04	147	88.44	9.72	9.53	−0.19	1.05	11.07
A-2018	01	155	88.39	5.34	5.30	−0.04	0.49	9.16
	02	155	89.03	7.49	7.47	−0.02	0.65	8.65
	03	155	91.61	6.08	6.06	−0.02	0.48	7.90
	04	155	88.39	8.66	8.75	0.09	1.08	12.40
	05	153	91.61	6.40	6.40	0.00	0.42	6.64
B-2018	01	153	92.81	6.33	6.29	−0.04	0.56	8.91
	02	152	86.18	10.13	9.98	−0.15	1.04	10.44
	03	152	91.45	7.47	7.43	−0.04	0.69	9.34
	04	150	88.00	5.36	5.28	−0.08	0.41	7.69
	05	151	89.40	6.08	6.06	−0.02	0.61	10.10
C-2018	01	148	91.89	6.08	6.01	−0.07	0.32	5.40
	02	149	91.95	7.40	7.34	−0.06	0.43	5.79
	03	149	89.26	5.33	5.31	−0.02	0.39	7.42
	04	141	91.49	6.22	6.10	−0.12	0.33	5.33
	05	141	87.23	7.99	7.87	−0.12	0.45	5.66
A-2019	01	186	88.17	5.29	5.35	0.06	0.75	13.95
	02	187	89.30	5.99	6.07	0.08	0.84	13.86
	03	187	86.63	6.98	7.03	0.05	0.69	9.79
	04	178	NA	5.99	6.00	0.01	0.66	11.05
	05	179	NA	8.69	8.66	−0.03	0.78	8.98
B-2019	01	180	86.11	5.29	5.24	−0.05	0.43	8.18
	02	179	87.15	5.99	5.95	−0.04	0.54	9.14
	03	180	90.56	7.16	7.14	−0.02	0.63	8.85
	04	180	91.67	5.43	5.43	0.00	0.49	9.03
	05	180	90.00	5.99	5.97	−0.02	0.52	8.75
C-2019	01	187	87.17	5.30	5.35	0.05	0.82	15.40
	02	187	89.84	5.99	6.03	0.04	0.91	15.06
	03	187	89.84	7.07	7.12	0.05	0.90	12.59
	04	187	86.10	5.40	5.46	0.06	0.85	15.66
	05	187	89.30	5.99	6.05	0.06	0.86	14.21
A-2020	01	230	86.09	8.03	8.03	0.00	0.65	8.10
	02	230	84.35	6.44	6.37	−0.07	0.56	8.81
	03	230	79.57	5.88	5.78	−0.10	0.51	8.80
	04	230	81.30	6.44	6.37	−0.07	0.58	9.09
	05	230	82.61	5.43	5.35	−0.08	0.48	8.90
B-2020	01	233	84.98	6.44	6.43	−0.01	0.59	9.14
	02	233	82.83	7.24	7.23	−0.01	0.63	8.65
	03	233	85.84	6.44	6.45	0.01	0.59	9.18
	04	233	84.98	8.62	8.74	0.12	0.76	8.72
	05	233	88.84	5.91	5.87	−0.04	0.42	7.21
C-2020	01	231	92.21	7.13	7.25	0.12	0.49	6.76
	02	231	88.93	10.39	10.59	0.20	0.66	6.23
	03	231	92.21	6.32	6.41	0.09	0.34	5.30
	04	231	91.39	6.32	6.41	0.09	0.54	8.42
	05	231	88.48	5.54	5.63	0.09	0.37	6.57

NA; Not analyzed.

4. Discussion

HbA1c measurement is important for the diagnosis and monitoring of diabetes. The American Diabetes Association (ADA) recommended a diagnostic cutoff value of 48 mmol/mol (6.5% HbA1c) for diabetes, and the World Health Organization (WHO) has

Table 3
Comparison of mean HbA1c values between each method and assigned value of EQA samples during 2016–2020 (54 samples).

Assay methods	N	Mean HbA1c assigned value (%)	Mean HbA1c value (%)	Relative bias (%)	SD	% CV	*P*-value
Immunoassay	54	6.69	6.71	−3.05 to 4.54	0.21–0.94	3.15–15.52	0.248
Enzymatic method	54	6.69	6.69	−5.97 to 4.23	0.35–1.91	5.74–21.69	0.745
High performance liquid chromatography	54	6.69	6.40	−11.13 to 1.90	0.15–0.76	2.59–12.42	<0.001
Low pressure liquid chromatography	12	6.72	5.41	−44.87 to −3.85	NA	NA	0.001
Boronate affinity chromatography	54	6.69	6.90	−21.25 to 31.19	0.24–2.70	3.75–36.64	0.025

NA; Not analyzed.

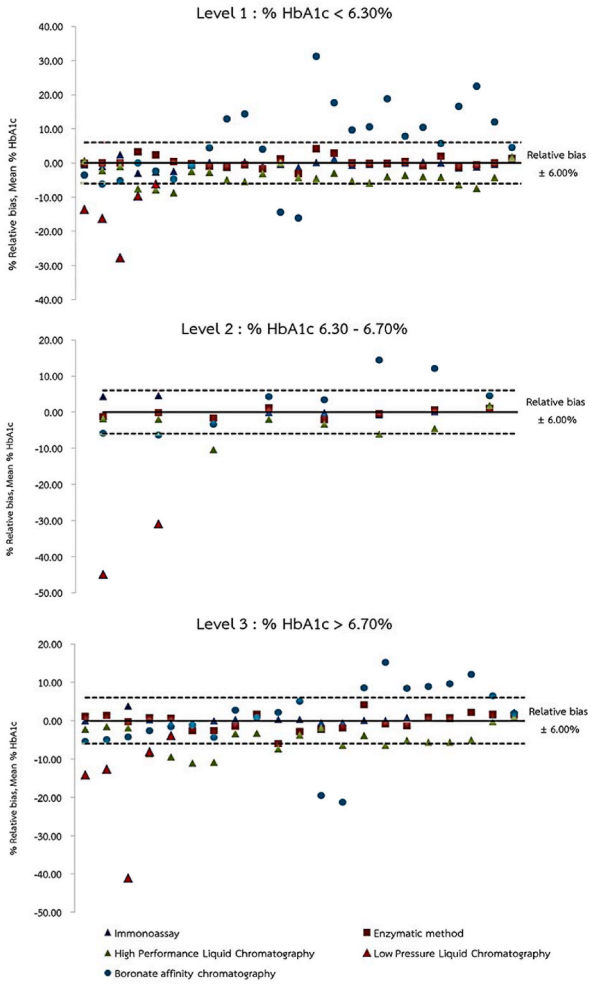

Fig. 2. Bias and variability from the IFCC assigned value cassified by assay methods. Level 1 (N = 25 samples), level 2 (N = 8 samples), level 3 (N = 21 samples).

published guidelines for using HbA1c in the diagnosis of diabetes mellitus [10–12].

The National External Quality Assessment Scheme for HbA1c has been established since 2016 [13]. We provided an accuracy-based EQA program using whole blood samples and the value assigned by the IFCC reference system to investigate the performance of HbA1c assays in the country. The EQA samples in our program had HbA1c values ranged from 4.90 to 10.59% covering normoglycemia, prediabetes, and diabetes. This approach allowed the laboratories participating in the proficiency testing to monitor the testing quality of all critical HbA1c values, including diabetes risk detection, diagnosis, and treatment monitoring [14]. The best approach of the EQA program for HbA1c is the use of whole blood samples and comparison to the target values assigned by using the IFCC reference

Table 4
The percentage relative bias and variability classified by assay methods during 2016–2020.

Assigned IFCC value	Number of participants (Min-Max)	Mean %HbA1c	Relative bias	SD	% CV
Immunoassay					
<6.30%	(83–166)	(4.95–6.14)	(-3.05 to +2.31)	(0.21–0.94)	(3.54–15.52)
6.30–6.70%	(83–166)	(6.3–6.82)	(-1.64 to +4.54)	(0.24–0.51)	(3.72–8.01)
>6.70%	(83–166)	(6.71–10.62)	(-1.40 to +3.84)	(0.22–9.39)	(3.15–9.39)
Enzymatic method					
<6.30%	(1–59)	(4.97–6.16)	(-3.22 to +4.08)	(0.35–1.01)	(5.74–16.89)
6.30–6.70%	(2–59)	(6.2–6.5)	(-2.05 to +1.31)	(0.38–0.85)	(5.95–13.44)
>6.70%	(1–59)	(6.81–10.55)	(-5.97 to +4.23)	(0.54–21.69)	(6.73–21.69)
High Performance Liquid Chromatography					
<6.30%	(7–31)	(4.91–5.95)	(-8.88 to +1.44)	(0.15–0.45)	(2.6–8.87)
6.30–6.70%	(7–31)	(5.75–6.44)	(-10.32 to +1.90)	(0.16–0.69)	(2.59–11.22)
>6.70%	(7–31)	(6.12–10.49)	(-11.13 to +0.96)	(0.18–12.42)	(2.77–12.42)
Low Pressure Liquid Chromatography					
<6.30%	1	(4.00–5.00)	(-6.19 to −27.8)	NA	NA
6.30–6.70%	1	(3.60–4.50)	(-30.88 to −44.87)	NA	NA
>6.70%	1	(5.20–8.10)	(−3.85 to −41.04)	NA	NA
Boronate affinity chromatography					
<6.30%	(1–7)	(4.56–7.04)	(-16.21 to +31.19)	(0.24–2.44)	(3.75–35.2)
6.30–6.70%	(2–7)	(6.1–7.37)	(-6.30 to +14.44)	(0.26–2.7)	(3.94–36.64)
>6.70%	(1–7)	(5.96–10.59)	(-21.25 to +15.22)	(0.42–2.39)	(3.97–27.31)

NA; Not analyzed.

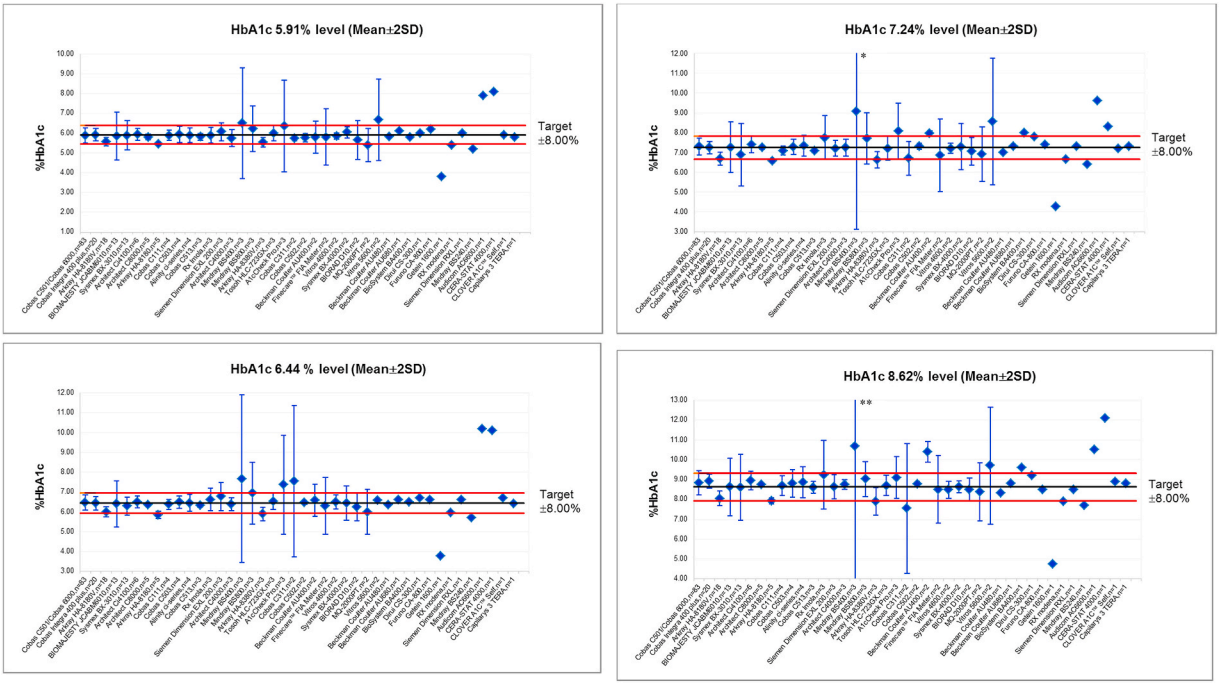

Fig. 3. Bias and Variability from the IFCC Target classified by assay methods in 2020. Target is assigned value with acceptable limit (±8.00%). ⊢ is mean±2SD of participant using each method.* 2SD = ±5.96%HbA1c ** 2SD = ±7.52%HbA1c.

procedure [15,16] and NGSP certified method [2,6,17]. Comparing their results and the targeted values, the participating laboratory could realize their HbA1c assays performance. In addition, our program also provides consultation from reference laboratories to the participants to improve and correct their testing.

As the noncommutability bias of lyophilized EQA materials for HbA1c was demonstrated [18], the use of commutable EQA materials improves in the evaluation of analytical performance among participating laboratories. Whole blood used in our EQA program is more commutable than other sample types [19,20]. In addition, to ensure the stability of the fresh blood, a proper logistic arrangement with the cold-chain condition was scheduled in advance, and samples arrived within a time frame of 24 h.

For the efficient HbA1c laboratory evaluation, it is essential to tighten the acceptable criteria. In 2016–2017, we assessed the performance of HbA1c assays with acceptance limits of ±10% difference compared to the assigned value and reduced to ±8% in 2021.

The application of more stringent acceptable criteria will improve laboratory performance in the HbA1c analysis. In 2007, a College of American Pathologists (CAP) survey in the United States began using accuracy grading with a permissible limit of 15% of a target value; this limit was reduced to 6% in 2020 [21]. This limit varies across Europe, ranging from ±5% in Scandinavia to ±8% in Belgium, the Netherlands, and Luxemburg, and ±18% in Germany [20].

The pass rates of our participating laboratories during 2016–2020 were 72–88%. The assay methods in our EQA program included both certified and noncertified NGSP methods.

The overall %CV ranged from 4.46 to 15.66%. The recommended interlaboratory HbA1c CV target was <3.5% [10]. In 2016, EQA in Germany, Belgium and the Netherlands using fresh whole blood samples with IFCC reference system target values showed between-laboratory CV of 4.1% in overall assay methods. The CAP survey in 2020, between-laboratory CV ranged from 0.7% to 5.1% [21]. The high CV in our survey could be attributed to the results of some assay methods having a bias with frozen EQA samples. The POCT has unacceptable values, probably from frozen sample, not commutable [22], the POCT results will compare with peer group.

During the 2016–2020 survey, the mean percentage bias based on the total data of all participating laboratories was −0.19 - 0.20%, with a SD of 0.30–1.08. It is likely that the bias and variability of HbA1c testing were due to assay methods bias. The HPLC ($P < 0.001$) were both significantly biased from the assigned value. The assay methods used in Thailand were mainly similar to those used in the European market [19,23]. Positive bias for HPLC assays was also observed in CAP surveys [24] and other studies [20,25]. The laboratory should avoid using instruments with a high CV and a large bias. Tightening the acceptability limit to ±8% may be reasonable and allow for more accurate identification of poor performing laboratories and diagnostic devices. EQA schemes are a key tool for improving accuracy in individual laboratories, and manufacturers. The limitations of test principles and instruments may result in inaccurate results of HbA1c analysis.

Participant laboratories where performance evaluations were not passed should investigate the source of the errors and improve their performance. HbA1c testing errors can occur at any stage, including the pre-analytical, analytical, and post-analytical phases [13]. Participant laboratories that were not passed should investigate the source of the errors and correction; therefore EQA program is a tool for improve laboratory performance.

5. Conclusion

We organized an EQA program using whole blood to investigate the performance of HbA1c assays across the country. Participating in the EQA program is an effective educational tool for monitoring the quality of testing systems and laboratories. When clinical laboratories, manufacturers, and EQA agencies combine efforts, the analytical performance of HbA1c assays can be significantly improved for the benefit of patients with diabetes mellitus.

Author statement

Copyright Assignment: The undersigned authors transfer all copyright ownership of this manuscript to The Japanese Respiratory Society, in the event the work is published. The undersigned authors warrant the article is original, does not infringe upon any copyright or other proprietary right of any third party, is not under consideration for publication by any other journal, and has not been published previously. The authors confirm that they have reviewed and approved the final version of the manuscript.

Institutional Committee Approval: Submissions must comply with the following policies: 1) Research involving human subjects should be conducted in conformity with the Declaration of Helsinki, and should be certified that ethical and humane principles of research have been followed: 2) Freely-given informed consent from the subjects or patients must be obtained: 3) Research involving animals should be conducted in conformity with the various laws about prevention of cruelty to animals.

Patients Permission: A letter of permission must be obtained from the subjects and patients no matter if the article appears in the published journal or in the online journal.

Acknowledgments

This study was supported by a research grant provided by the Department of Medical Sciences, Ministry of Public Health, Thailand. We want to thank the research team from the Transfusion transmitted pathogen section, National Institute of health, for technical support.

References

[1] C. Weykamp, HbA1c: a review of analytical and clinical aspects, Ann Lab Med 33 (2013) 393–400.
[2] F. Braga, M. Panteghini, Standardization and analytical goals for glycated hemoglobin measurement, Clin. Chem. Lab. Med. 51 (2013) 1719–1726.
[3] Clinical and Laboratory Standards Institute (CLSI), in: Using Proficiency Testing to Improve the Clinical Laboratory; Approved Guideline, second ed., Clinical and Laboratory Standards Institute, Pennsylvania, USA, 2007. CLSI document GP27-A2.
[4] G.J. Kost, A. Kanoksilp, D.M. Mecozzi, R. Sonu, C. Curtis, J.N. Yu, Point-of-need hemoglobin A1c for evidence-based diabetes care in rural small-world networks: Khumuang Community Hospital, Buriram, Thailand, Point Care 10 (2011) 28–33.
[5] M.J. O'Brien, D.B. Sacks, Point-of-Care hemoglobin A1c, JAMA 322 (14) (2019) 1404–1405.
[6] R.R. Little, C.L. Rohlfing, HbA1c standardization: background, progress and current issues, Lab medicine 40 (2015) 368–373.
[7] ISO/IEC 17043: 2010, Conformity Assessment-General Requirements for Proficiency Testing, ISO, Geneva, Switzerland, 2010.
[8] ISO 13528: 2015, Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons, ISO, Geneva, Switzerland, 2015.
[9] College of American Pathologists (CAP) survey data, Available at: http://www.ngsp.org/. (Accessed 18 April 2022).

[10] D.B. Sacks, M. Arnold, G.L. Bakris, D.E. Bruns, A.R. Horvath, M.S. Kirkman, et al., Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus, Clin. Chem. 57 (2011) e1–47.
[11] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diabetes Care 1 (33Suppl) (2010) S62–9.
[12] World Health Organization, Use of Glycatedhaemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation, WHO, Geneva, 2011, pp. 1–25.
[13] S. Suparak, M. Kunakorn, B. Pratumvinit, C. Setthaudom, B. Sriwanthana, National external quality assessment scheme for HbA1c: evaluation of performance using assigned values, J. Med. Tech. Assoc. Thailand 45 (3) (2017 Dec) 6194–6208.
[14] International expert committee report on the role of the A1C assay in the diagnosis of diabetes, Diabetes Care 32 (2009) 1327–1334.
[15] C. Weykamp, W.G. John, A. Mosca, T. Hoshino, R. Little, J.O. Jeppsson, I. Goodall, K. Miedema, G. Myers, H. Reinauer, D.B. Sacks, R. Slingerland, C. Siebelder, The IFCC reference measurement system for HbA1c: a 6-year progress report, Clin. Chem. 54 (2) (2008 Feb) 240–248.
[16] N. Clouet-Foraison, P. Gillery, DelatourV, Achieving comparability with IFCC reference method for the measurement of hemoglobin A1c by use of an improved isotope-dilution mass spectrometry method [letter], Anal. Bioanal. Chem. 409 (24) (2017 Sep) 5789–5790.
[17] Consensus Committee, Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American diabetes association, European association for the study of diabetes, international federation of clinical Chemistry and laboratory medicine, and the international diabetes federation, Diabetes Care 30 (2007) 2399–2400.
[18] V. Delatour, N. Clouet-Foraison, S. Jaisson, P. Kaiser, P. Gillery, Beware of noncommutability of external quality assessment materials for hemoglobin A1c, Clin. Chem. 66 (2) (2020 Feb 1) 390–391.
[19] P. Kaiser, M. Spannagl, C. van Campenhout, Y. Lenga, C. Siebelder, C. Weykamp, HbA1c: EQA in Germany, Belgium and The Netherlands using fresh whole blood samples with target values assigned with the IFCC reference system, Clin. Chem. Lab. Med. 54 (2016) 1769–1775.
[20] A. Mosca, R. Paleari, A. Carobene, C. Weykamp, F. Ceriotti, Performance of glycated hemoglobin (HbA(1c)) methods evaluated with EQAS studies using fresh blood samples: still space for improvements, Clin. Chim. Acta 451 (2015) 305–309.
[21] College of American Pathologists (CAP) survey data, Available at: http://www.ngsp.org/CAPdata.asp. (Accessed 3 April 2021).
[22] E. Lenters-Westra, E. English, Are hemoglobin A1c point-of-care analyzers fit for purpose? The story continues, Clin. Chem. Lab. Med. 59 (4) (2020 Nov 2) 765–774.
[23] Weykamp, on behalf of the group of the EurA1c study group, EurA1c: the European HbA1cTrial to investigate the performance of HbA1c assays in 2166 laboratories across 17 countries and 24 manufacturers using the IFCC Model for Quality Targets, Clin. Chem. (2018). In press.
[24] College of American Pathologists (CAP) survey data, Available at: http://www.ngsp.org/CAPdata.asp. (Accessed 3 May 2018).
[25] S.E. Manley, L.J. Hikin, R.A. Round, et al., Comparison of IFCC-calibrated HbA(1c) from laboratory and point of care testing systems, Diabetes Res. Clin. Pract. 105 (2014) 364–372.