An evaluation of the Quo-Test® performance against NGSP criteria and sigma-metrics

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Diabetes Care

Quo-Test

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Introduction

The first evaluation of the Quo-Test® was performed by the ERL (European Reference Laboratory) in 2009[1]. This evaluation was not completed as a number of small technical issues with the analyzer became apparent early in the course of the project.

A year later, in 2010, a second evaluation was performed which showed an undesirable lot-lot variability[2]. A third evaluation[3] in 2014 identified a difference in results between frozen and fresh patient samples. Because Quo-Test® was calibrated using frozen IFCC secondary reference material, the fresh patient samples showed a significant negative bias.

Following cooperation between the European Reference Laboratory for Glycohemoglobin and Quotient Diagnostics Ltd, an EKF Diagnostics company, Quo-Test® was submitted for further evaluation.

Methods

To get an overall impression of performance before starting the CLSI EP-5 and EP-9 protocols, 12 fresh patient samples were run in duplicate. Values were assigned to the fresh patient samples by the three Secondary Reference Method Procedures (SRMPs) detailed below. Precision was also calculated by analyzing the samples in duplicate. The results were satisfactory to allow the continuation of the evaluation.

The CLSI EP-5 protocol was used to further investigate assay imprecision (duplicate measurements twice per day on two patient samples for 20 days). Aliquots were made from the patient samples and stored at minus 80°C degrees until analysis. CVs were also calculated on the basis of the duplicates of the fresh patient samples in the EP-9 protocol. The CLSI EP-9 protocol was performed twice with two different reagent lot numbers, and the data was used to investigate the bias between the Quo-Test® and the three SRMPs (n= 48 (normally n=40), five days, duplicate measurements).
The data was also used to calculate the NGSP certification criteria. NGSP certification requires that, beginning in January 2014, 37 of 40 results must be within 6% (relative) of an individual NGSP SRMP to pass.

HbA1c value determination of the patient samples was performed with three certified SRMPs:

- Roche Tina-quant Gen.2 HbA1c on Integra 800,immunoassay, IFCC and NGSP certified (Roche Diagnostics);
- Premier Hb9210, affinity chromatography HPLC, IFCC and NGSP certified (Trinity Biotech); and
- Tosoh G8, cation-exchange HPLC, IFCC certified (Tosoh Bioscience).

To check overall calibration and bias independently of the chosen SRMP, the results of the Quo-Test® instruments in the EP-9 procedure were compared with the mean of the three SRMPs, and medical decision point (MDP) analysis was performed at an HbA1c value of 48 mmol/mol (6.5% Diabetes Control and Complications Trial (DCCT) units) and 75 mmol/mol (9.0% DCCT units). When the two methods are statistically identical, the 95% CI for each y MDP includes the corresponding x MDP.

Interference from common Hb variants HbAS, HbAC, HbAD, HbAE, HbAJ, increased A2 (β-thalassemia), and HbF was investigated by the Quo-Test®. Five samples of each variant with different HbA1c values were analyzed in one day.

Results

Table 1 shows the results of the EP-5 protocol and the CVs on the basis of the duplicate samples analyzed in EP-9.

Table 1: Imprecision results based on EP-5 and on the duplicates in EP-9

	CV (%) SI units	CV (%) DCCT units
Quo-Test (EP-5)	3.3 (49 mmol/mol)	2.2 (6.6%)
Quo-Test (EP-5)	1.8 (75 mmol/mol)	1.3 (9.0%)
Lot number 1*	1.9	1.3
Lot number 2*	1.2	0.9

* Based on duplicates in EP-9

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Table 2 shows NGSP certification pass/fail criteria with respect to the results of the EP-9 protocol performed with fresh patient samples.

Table 2: EP-9 results in DCCT units and calculations of NGSP certification criteria

Deming regression lines	Lot number 020268	Bias	SEE	out ± 6% SRM	NGSP criteria
Quo-Test (Y) vs Premier (X)	Y=0.96X + 0.33	0.07	0.13	0	Pass
vs Tina-quant (X)	Y=0.95X + 0.43	0.09	0.16	2	Pass
vs Tosoh G8 (X)	Y=0.93X + 0.58	0.06	0.20	4	Pass*
Deming regression lines	**Lot number 020269**	**Bias**	**SEE**	**out ± 6% SRM**	**NGSP criteria**
Quo-Test (Y) vs Premier (X)	Y=1.00X + 0.14	0.12	0.14	0	Pass
vs Tina-quant (X)	Y=0.99X + 0.24	0.14	0.18	1	Pass
vs Tosoh G8 (X)	Y=0.96X + 0.41	0.11	0.20	3	Pass

*37 of 40 results need to be within 6% (relative) of an individual NGSP SRMP to pass certification. In this study 48 samples were analyzed instead of 40.

Figure 1 shows the EP-9 results compared to the mean of the three SRMP. The mean bias of the two lot numbers used compared to the mean of the three SRMP is < 2 mmol/mol (bias lot number 020268 is 0.6 mmol/mol and lot number 020269 is 1,1 mmol/mol).
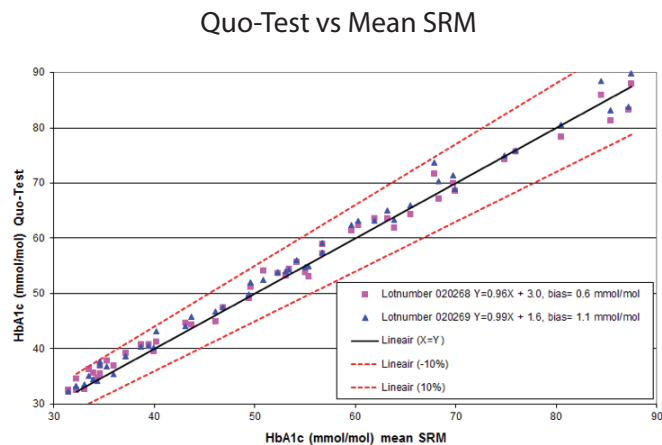

Quo-Test vs Mean SRM

Figure 1: Quo-Test vs mean Premier Hb9210, Tosoh G8 and Roche Tina-quant on Integra 800.

Table 3 shows the results of the medical decision point analysis of 48 mmol/mol and 75 mmol/mol compared to the mean of the three SRMP and the individual SRMP.

Table 3: Medical decision point of 48 mmol/mol and 75 mmol/mol (in brackets the 95% CI) compared to the different reference methods

	Lot number 020268		Lot number 020269	
Mean SRM	48.8 (48.5–49.2)	74.6 (74.1–75.2)	49.2 (48.9–49.5)	75.9 (75.4–76.5)
Tosoh G8	48.9 (48.3–49.4)	73.7 (72.9–74.4)	49.2 (48.7–49.7)	75.0 (74.3–75.7)
Roche Tina-quant Integra 800	48.8 (48.4–49.2)	74.7 (74.1–75.3)	49.1 (48.7–49.5)	76.1 (75.5–76.7)
Premier Hb9210	49.0 (48.7–49.3)	75.1 (74.6–75.6)	49.3 (49.0–49.7)	76.5 (76.0–77.0)

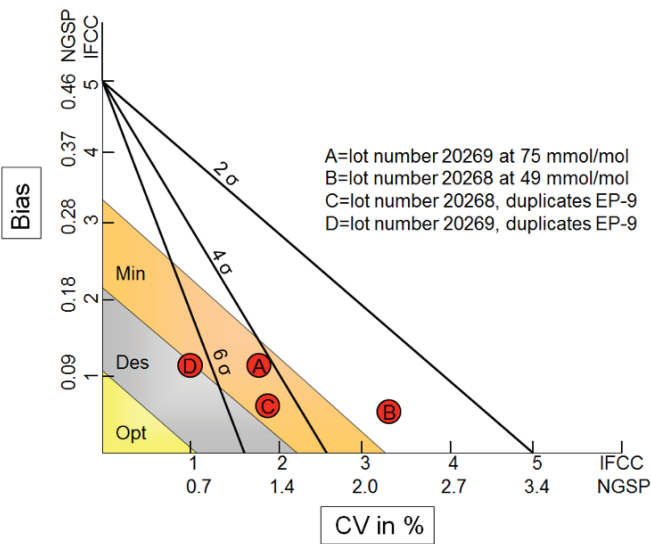Figure 2 shows the analytical performance of the Quo-Test in sigma metrics.

A=lot number 20269 at 75 mmol/mol
B=lot number 20268 at 49 mmol/mol
C=lot number 020268, duplicates EP-9
D=lot number 020269, duplicates EP-9

Figure 2: Analytical performance of the Quo-Test in sigma metrics.

*Based on duplicates in EP-9

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Figure 3 shows the results of fresh normal non-variant samples (n=48) and frozen Hb-variants samples (n=5 per Hb-variant). The mean relative difference of the Hb-variants measured in SI units (mmol/mol) compared to the assigned value was for HbAS 1.1%, HbAC 1.2%, HbAD 5.1%, HbAE 9.2%, HbAJ 0.7% elevated A2 5.3% and HbF -7.0%. The percentage HbF in the samples were 3.2%, 4.6%, 8.6%, 15% and 18%. These results have not been corrected for the bias with frozen normal non-variant samples.
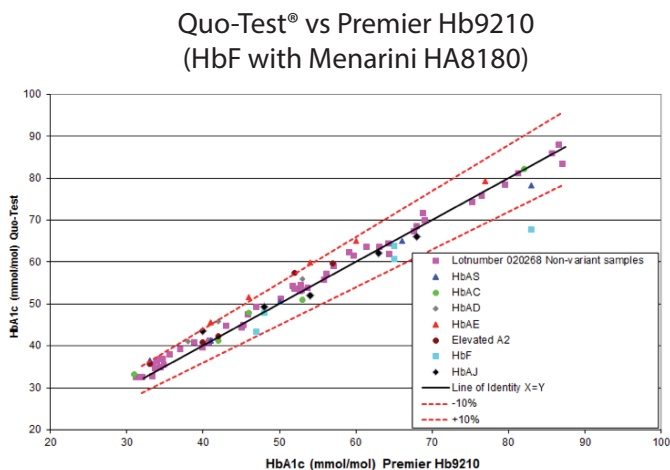
Quo-Test® vs Premier Hb9210 (HbF with Menarini HA8180)

Figure 3: Interference of Hb-variants with the Quo-Test

Discussion

The CV in the EP-5 protocol at an HbA1c value of 49 mmol/mol is a little (3.3% in SI units and 2.2% in DCCT units) above the criteria of <3% in SI units (<2% in DCCT units). However, the CVs at an HbA1c value of 75 mmol/mol and the CVs of the duplicates in the EP-9 were well within the acceptable criteria.

The calibration of the Quo-Test® is sufficient which was confirmed by passing the NGSP criteria compared with the three SRMPs. The mean bias compared with the mean of the SRMPs was well within the acceptable criteria of <2 mmol/mol. Medical decision point analysis at an HbA1c value of 48 mmol/mol and 75 mmol/mol showed a significant statistical difference compared with the mean of the SRMP and the individual SRMP.

However, clinically seen, the differences were small and therefore acceptable. The combined performance in sigma metrics is very good. Sigma is >2 at 48 mmol/mol and at a HbA1c value of 75 mmol/mol > 4. If the CVs of the duplicates are examined then it is apparent that one lot number has a sigma >6; this is a class-leading level of performance.

From the previous study we know that the Quo-Test® had a negative bias of approximately 2 mmol/mol with fresh patient samples because in the past frozen IFCC secondary reference material was used to calibrate the Quo-Test® and this material appeared not to be commutable with the Quo-Test® methodology. To investigate the interference of Hb-variants the study used frozen samples. Remarkably, the Quo-Test® showed no interference of the common Hb-variants HbAS, HbAC, HbAD, HbAE, HbAJ, elevated A2 (β-thallasemia) and HbF <8.6% (figure 2). The main negative relative difference of the Hb-variants compared with the 2014 study was that this study compensated by the adjusted calibration with fresh patient samples, except for HbAE. In the 2014 study the Quo-Test® had a positive relative difference of HbAE samples compared with the reference method but now, due to the adjusted calibration, the mean relative difference was 9.2%.

Conclusions:

The Quo-Test® met the generally accepted performance criteria for HbA1c.

References

1. Lenters-Westra E, Slingerland RJ. Six of eight Hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. Clinical Chemistry 2010;56:44-52.
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4. Weykamp C, John G, Gillery P, English E, Ji L, Lenters-Westra E, Little RR, Roglic G, Sacks DB, Takei I. Investigation of 2 Models to Set and Evaluate Quality Targets for HbA1c: Biological Variation and Sigma-Metrics. Clinical Chemistry 2015;61:752-9.

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