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External Validation and Lay User Field Studies of Afinion™ HbA1c using the Afinion™ AS100 Analyzer

Afinion™ AS100 Analyzer System

Afinion™ AS100 Analyzer (Figure 1) is a small, bench top, multi-assay analyzer for in vitro diagnostic Point of Care Testing. The Analyzer with the single packed Test Cartridges constitutes the Afinion™ AS100 Analyzer System. Assayed Afinion™ control materials are available for routine quality control of the Analyzer System.

The Afinion™ technology enables application of different assay principles, which allows for analysis of a variety of biochemical parameters in human blood or urine.

A simple sampling procedure, easy touch screen user interface, no manual calibration, no chemistry handling or manual calculation of results makes the Afinion™ Analyzer System suitable for both laboratory and non-laboratory users.

Figure 1. *Afinion™ AS100 Analyzer System.*

Afinion™ HbA1c – a CLIA waived test

Afinion™ HbA1c is one of the first tests developed for the Afinion™ AS100 Analyzer. Measurement of HbA1c with Afinion™ AS100 Analyzer demonstrates robustness, simplicity and insignificant risk of erroneous results. Afinion™ HbA1c is waived under the Clinical Laboratory Improvement Amendment of 1988 (CLIA`88).

Afinion™ HbA1c measures glycated hemoglobin in human blood as a marker of long-term metabolic control in persons with diabetes mellitus. Afinion™ HbA1c is particularly suitable for routine follow-up of diabetic patients in the physicians' office laboratories and diabetes clinics.

Automated boronate affinity assay

The Afinion™ HbA1c Test Cartridge contains the reagents necessary for one sample analysis, and is to be disposed after use. The blood sample is collected with the integrated sampling device before the cartridge is placed in the Analyzer. The sample is then automatically diluted and mixed with a buffer that lyses the erythrocytes and precipitates the hemoglobin. This sample mixture is transferred to a blue boronic acid conjugate, which binds to the glycated hemoglobin. The reaction mixture is then soaked through a filter membrane. All precipitated hemoglobin, conjugate-bound and unbound (i.e. glycated and non-glycated hemoglobin), remains on the membrane while the excess of conjugate is removed with a washing reagent.

The Analyzer evaluates the precipitate on the membrane. The ratio between the measured blue (glycated hemoglobin) and the red (total hemoglobin) color intensities is proportional to the % HbA1c displayed on the Analyzer screen.

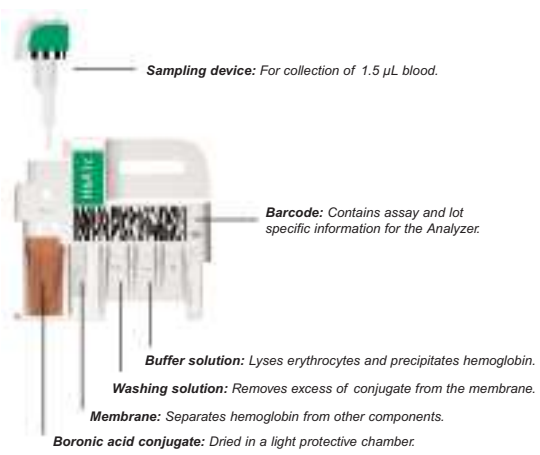

Figure 2. *Afinion™ HbA1c Test Cartridge.*

Safe and simple operation

To run an Afinion™ HbA1c test, no additional instruments are required besides standard capillary or venous puncture equipment. Printer and barcode-scanner (for entering sample ID) can optionally be connected.

The Test Cartridge is placed in the cartridge chamber and the analysis starts by closing the lid manually. Once loaded with a Cartridge, the Afinion™ Analyzer represents a true walk-away system. After analysis the result is stored and displayed on the screen. When the result is accepted or transferred to a connected printer or data system, the lid opens automatically and the used Cartridge can be removed and discarded.

Safety
Afinion™ is a closed system where no reagents, sample residuals or hazardous components of the Test Cartridge or Analyzer are exposed to the user. With an optimal design and handling of Analyzer and Test Cartridge, the risk of contamination from biological or chemical sources is reduced to a minimum. The sampling device prevents user contact with the capillary at blood collection. After completed analysis, the Test Cartridge can be safely removed from the Analyzer and immediately placed in a suitable waste container.

Speed
The Afinion™ HbA1c analysis of a capillary or venous blood sample takes just 3 minutes. The short test time is crucial for POC testing, as the test result can be presented for the doctor during the patient consultation and taken into consideration for immediate diagnosis, follow up and treatment.

Simplicity
The touch screen of the Analyzer provides a simple graphical user interface (GUI). Colored icons are used for language-independent touch buttons while the local language can be chosen for text messages.

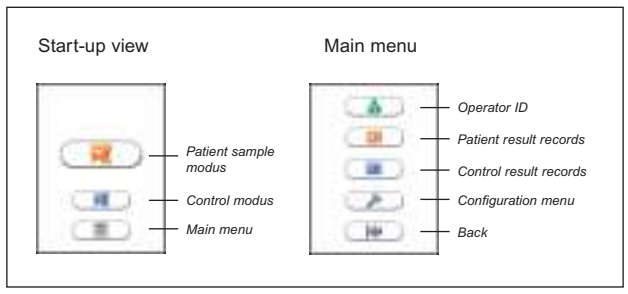

Figure 3. *Examples of screen views and symbols used in the GUI.*

Step-by step Procedure

Collect sample	Analyze sample
 *1. Open foil pouch.*	 *5. Insert the Test Cartridge.*
 *2. Pull up the sampling device.*	 *6. Close the lid manually. Enter Patient ID.*
 *3. Touch the surface of the blood drop and fill the capillary to the end.*	 *7. Record the result when it appears on the screen.*
 *4. Insert the sampling device.*	 *8. Remove and discard the cartridge.*

Insignificant risk of erroneous results

Accurate test results are secured through the standardization of the Afinion™ assays, the calibration of each Cartridge lot, the calibration of the Analyzer and the internal process control (the self-test and the fail-safe system). To prevent any mix-up of data, the Analyzer also has a function for entering (by manual typing or barcode reading) the operator- and patient identification, and thus secures the link between operator, patient and test result. All data are stored and can be viewed, printed or transferred to an independent data connectivity system. The Afinion™ Controls also provide for external quality control.

International standardization
Afinion™ HbA1c is traceable to the IFCC Reference Method for Measurement of HbA1c (International Federation of Clinical Chemistry and Laboratory Medicine). HbA1c values are reported according to NGSP (National Glycohemoglobin Standardization Program, USA) recommendations at DCCT level (Diabetes Control and Complications Trial). Afinion™ HbA1c meets the performance standards established by NGSP.

No interference from hemoglobin variants and derivates
The Hb-variants HbAC, HbAD, HbAE, HbAJ, HbAS, HbF and carbamylated Hb do not interfere with analysis of % HbA1c on the Afinion™ AS100 Analyzer according to the ERL Manufacturer Check Up certification protocol criteria.

Calibration without user intervention
During manufacturing, the Analyzers are calibrated against a reference system to ensure that all Analyzers operate within identical tolerance limits. Test specific calibration data are established for each lot of Test Cartridges and then stored in the barcode label. When the Cartridge enters the Analyzer, the integrated camera reads the barcode. The calibration data for the actual lot are transferred to the Analyzer and used for calculating the results. Calibration by the operator is thus not required.

Extensive fail-safe system prevents display of erroneous results
A self-test is performed during start-up of the Analyzer to validate the hardware and software integrity, the cartridge transport system, the liquid transport system and the camera vision system. If the self-test fails at any point, an information code will be displayed. If the Analyzer remains on for a longer period, it will automatically restart once a day to ensure that the self-test is done regularly.

Fail-safe mechanisms are included to secure safe analysis and prevent erroneous results. During analysis, numerous parameters are controlled. The image based fail-safes include inspections of the barcode and capillary, geometrical and mechanical inspections and reagent volume inspections. If errors occur, the analysis will be aborted and no result will be displayed.

Afinion™ HbA1c Control for external QC
Stabilized liquid controls are available for the Afinion™ HbA1c test. Measured values within the stated acceptable ranges confirm that the Afinion™ Analyzer System works properly and provides reliable results. The procedures for analyzing control and patient samples are identical. By selecting the control mode from the screen, the results are stored in a separate control record.

Lay user study demonstrating precision, simplicity and low risk of erroneous results

Objective
The objective for this study was to demonstrate that:

- The Afinion™ HbA1c test system provides precise results in the hands of the intended users, here defined as operators with no laboratory education or previous experience with the Afinion™ Analyzer System.
- The Afinion™ HbA1c test system has an insignificant risk of erroneous results.
- The Afinion™ HbA1c test system is easy to use, and the labeling and instructions fulfils the criteria for CLIA waived point-of-care testing.

Study sites and operators
The participants were selected from three sites in Oslo, Norway, one high school and two workplaces. Education level was from primary school to university. None of the included 68 participants had laboratory education or previous experience with the Afinion™ Analyzer System. Age distribution was from 16 to >60 years.

Procedure
For the precision study, six Afinion™ AS100 Analyzers, one production lot of Afinion™ HbA1c and three blood samples were used (Table 1).

Table 1. *Samples used for the precision study.*

Sample	Target value
Sample A (Afinion™ HbA1c Control C I)	6.0 % HbA1c
Sample B (Afinion™ HbA1c Control C II)	7.6 % HbA1c
Sample C (patient whole blood)	5.2 % HbA1c

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Each of the 68 participants analyzed the three samples once. The users were not coached or orally instructed in how to operate the Afinion™ Analyzer System. The performance was based only on printed supporting material written in English (Afinion™ User Manual, HbA1c Quick Guide and Package Insert), although the native language was Norwegian for 95% of the participants.

To demonstrate "insignificant risk of erroneous results", terminated runs and information codes displayed on the Analyzer screen were recorded and the participants had to take corrective actions according to instructions in the User Manual.

To demonstrate "simplicity", each participant was asked to answer a Lay user Questionnaire after performing the analyses.

Precision results – Lay user study

Mean % HbA1c, recovery (from the predetermined target values), the within-site and total precision for sample A, B and C were calculated from the results obtained by the 68 participants at the three study sites. The results are presented in Table 2.

Table 2. *Mean % HbA1c, recovery, within-site and total precision for sample A, B and C calculated from the results obtained at the three study sites.*

Sample	Site	Mean % HbA1c	Recovery %	Within-site CV (%)	Total mean % HbA1c	Total precision CV (%)
A	1	6.0	100	1.4	6.0	1.6
	2	6.0	100	1.7		
	3	6.0	100	1.7		
B	1	7.6	100	1.6	7.6	1.7
	2	7.7	101	2.0		
	3	7.6	100	1.6		
C	1	5.2	100	1.5	5.2	1.6
	2	5.2	100	1.4		
	3	5.2	100	1.9		

The accuracies of the results were evaluated against the Allowable Limits of Error (A.L.E.) or Tonks Limits. The calculation of the A.L.E. is based on the premise that one cannot distinguish properly between normal and abnormal values at the borderline of the normal range when errors are greater than one-quarter of this range.

$$\text{A.L.E. (in \%)} = \pm \frac{1/4 \text{ Normal range}}{\text{Mean of normal range}} \times 100\ \%$$

Based on a normal range of 4 - 6 % HbA1c and mean value of 5 % HbA1c, the A.L.E. for HbA1c measurements are defined as Mean ± 10 %. The calculated A.L.E. and total range obtained for each of the three samples are presented in Table 3.

Table 3. *The Allowable Limits of Error (A.L.E.) and the total ranges obtained for the three HbA1c samples.*

Sample	Target value (predetermined) % HbA1c	A.L.E. (Mean ± 10 %) % HbA1c	Total range (N = 68) % HbA1c
A	6.0	5.4 - 6.6	5.8 - 6.2
B	7.6	6.8 - 8.4	7.3 - 7.9
C	5.2	4.7 - 5.7	5.0 - 5.4

Lay user questionnaire

Each user was required to fill in a lay user questionnaire. The participants were asked if they ever had worked in a clinical laboratory setting, if they succeeded running the Afinion™ HbA1c test, if they intuitively understood the touch screen symbols and how they managed to navigate in the menu. They were also asked questions about the understanding of the printed information material.

According to the answers given by the operators in the questionnaire, none had any previous experience in laboratory work and were all qualified for inclusion in the study. The operators gave high scores on questions related to use of the Afinion™ AS100 Analyzer System. On a scale from 1- 4 (where 1 is low and 4 is high score), the scores were between 3.1 and 4.0. Intuitive understanding of the touch screen symbols achieved lowest score (3.1). However, some initial misinterpretation of the symbols had no impact on the operators' ability to handle the Analyzer, shown by high score in the remaining questions (3.8 - 4.0).

Terminated analyses due to errors

If an analysis was terminated due to an error detected by the Analyzer's fail-safe system, the operator was alerted by a displayed error code, and had to take corrective actions by using the provided information material; the HbA1c Quick Guide and the User Manual.

In this study, the detected errors were mainly caused by coagulated or hemolytic samples. Such user or sample errors may occur in clinical practice and it is thus important that the fail-safe system detects these failures and terminates the analyses. In this setting, these particular errors can be explained by the design of the study as the blood sample was collected from a drop on the surface of Para-film, imitating a finger prick blood drop for capillary sampling. Insufficient mixing of the blood in the tube, and pipetting from the bottom of the tube to the Para-film, gave a high concentration of red blood cells and a hemoglobin result above the hemoglobin measuring range. The operators realized that a more thorough mixing of the blood sample solved this problem. With a real finger prick sample this cause of error is not relevant.

Conclusion from lay user study

All test results were well within the calculated Allowable Limits of Error (Tonks Limits). The within-site CV of 1.4 - 2.0 % and the total CV of 1.6 - 1.7 %, can be considered as excellent based on the fact that the analyses were done by lay users with no previous experience in laboratory work. Recovery results, based on total mean from the three study sites, are 100 % for all three levels of HbA1c samples.

In the lay user questionnaire the operators gave high scores on four questions related to use of the Afinion™ HbA1c test system. The participants were quite satisfied with their own performance and also the understanding of the printed instructions, despite the foreign language used in this material.

From the lay user study we can thus conclude:

- The Afinion™ HbA1c assay is accurate and precise.
- The Afinion™ Analyzer System provides an insignificant risk of erroneous results due to a comprehensive fail-safe system that aborts the analysis and eject the test cartridge if an error is detected.
- Afinion™ HbA1c is easy to use and can be satisfactorily operated by a user with no laboratory education or previous experience with the Afinion™ Analyzer System.

Validation of Afinion™ HbA1c in Physician Office Laboratories (POL)

Objective

The objective for this study was to demonstrate that the Afinion™ HbA1c assay provides precise results in the hands of the intended users, here defined as personnel working in physician office labs.

Study sites and operators

Three physician office laboratories close to Oslo, Norway, were selected for the study. At least two operators at each site were involved in the study. The participating operators were familiar with point of care testing and used different POCT systems routinely.

Procedure

The precision study lasted for two weeks, using one lot Afinion™ HbA1c each week. Every day, for 10 days, six replicates of each of the three blood samples A, B and C (Table 4) were analyzed at the three study sites. The blood samples were stored refrigerated.

Table 4. *EDTA blood samples used for the precision study.*

Sample	Assigned value
Sample A	4.9 % HbA1c
Sample B	6.1 % HbA1c
Sample C	8.9 % HbA1c

For each sample, the following precision results were calculated:

- With-in day and within-site precision for both Afinion™ HbA1c lots using the NCCLS Guideline EP5-A estimates when performing more than two aliquots per day.
- Total precision, including the results from all three sites, for each Afinion™ HbA1c lot and for all sites and both lots.
- Average lot-to-lot variation.

Precision results – POL study

The within-day, within-site and total precision for sample A, B and C are presented in Table 5.

Table 5. *Mean % HbA1c, within-day and within-site precision (CV, %) for each Afinion™ HbA1c lot and each site. Total precision (CV, %) for both Afinion™ HbA1c lots, all sites.*

Sample	Lot	Site	Mean % HbA1c	Within-day CV (%)	Within-site CV (%)	Total CV (%)
A	1	1	5.0	1.7	1.9	2.3
		2	5.0	2.9	2.9	
		3	4.9	1.5	2.0	
	2	1	5.1	1.1	1.2	
		2	5.0	1.8	2.0	
		3	4.9	1.6	1.8	
B	1	1	6.2	2.4	2.4	2.3
		2	6.1	2.0	2.0	
		3	6.0	1.2	1.4	
	2	1	6.3	1.3	1.8	
		2	6.2	1.2	1.2	
		3	6.2	0.8	0.9	
C	1	1	9.1	1.3	1.4	2.3
		2	8.8	1.4	1.6	
		3	8.7	2.0	1.9	
	2	1	9.1	0.9	1.0	
		2	8.8	1.1	1.0	
		3	8.8	1.1	1.0	

Table 6 shows the average lot-to-lot variation calculated for each sample as:
Deviation (% HbA1c) = Mean % HbA1c Lot 2 - Mean % HbA1c Lot 1
Deviation (%) = (Mean % HbA1c Lot 2 - Mean % HbA1c Lot 1) x 100/ Mean % HbA1c Lot 1

Table 6. *Lot-to-lot variation of % HbA1c results from three study sites.*

Deviation Lot 2 - Lot 1	Sample A	Sample B	Sample C
Deviation (% HbA1c)	0.03	0.14	0.03
Deviation (%)	0.6	2.2	0.4

Conclusion from POL study

From the study performed in the Physician Office Laboratories, we can conclude that Afinion™ HbA1c fulfills the expectations to a precise Point of Care test:

- The within-day precision (CV) ranged from 0.8 to 2.9%.
- The within-site precision (CV), which also included the day-to-day variation, ranged from 0.9-2.9%.
- A total precision (CV) of 2.3 % was obtained with all three blood samples, tested over a period of 10 days at three study sites, using two lots of Afinion™ HbA1c and three Afinion™ AS100 Analyzers (one at each site).

Overall conclusion

The total precision with a CV of 2.3 % for the Afinion™ HbA1c assay when performed in a physicians office, according to the NCCLS protocol for assay performance, is well within the acceptance criteria for a Point of Care Test.

Regarding the lay user field study, both the precision results and the score on handling given by the lay users, are excellent. The results show that the Afinion™ AS100 Analyzer System is well qualified for use in any POC setting and also in CLIA Waived settings.

The US Food and Drug Administration (FDA) confirmed this statement by clearing the Afinion™ Analyzer System for CLIA Waived status in February 2006.

The Afinion™ Analyzer Point of Care System from Axis-Shield was selected as winner in the 2006 Medical Design Excellence Awards (MDEA) competition for In-Vitro Diagnostic Medical Devices.

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