Abbott

AFINION™ HbA1c Dx

Hemoglobin A1c test
For use with AFINION™ 2 and Alere Afinion™ AS100 Analyzer

Please consult the Afinion™ Analyzer User Manual for operation of the analyzer and general handling of the test cartridge.

Technical Support
The manufacturer provides a toll free line for technical support.
Call 1-866-216-9505 (available for use only in the United States of America)

REF 1116794

℞ Only IVD CE

1117105 Rev. A 2020/10

AFINION™ HbA1c Dx

For use with Afinion™ 2 and Alere Afinion™ AS100 Analyzer.

This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF including those with known Heredity Persistence of Fetal Hemoglobin. Refer to the Analytical specificity and Limitations sections in this package insert for details.

CLIA statement

Afinion HbA1c Dx is categorized as moderate complexity under the Clinical Laboratory Improvement Amendment of 1988 (CLIA'88).

PRODUCT DESCRIPTION

Intended use

Afinion HbA1c Dx is an *in vitro* diagnostic test for quantitative determination of glycated hemoglobin (% hemoglobin A1c, HbA1c) in human venous and capillary whole blood.

The test is to be used as an aid in the diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.

The measurement of % HbA1c is recommended as a marker of long-term metabolic control in persons with diabetes mellitus.

Summary and explanation of the test

The human erythrocyte is freely permeable to glucose. Within each erythrocyte a slow, continuous, non-enzymatic process between hemoglobin A and various sugars takes place. The product formed is known as glycated hemoglobin, or glycohemoglobin[1].

An international Expert Committee has concluded that measurements of HbA1c can be used to diagnose diabetes mellitus and identify patients who may be at risk of developing diabetes[2].

The chronic elevated blood sugar level of persons with diabetes mellitus will over time cause damage to the small vessels of the body. This damage develops slowly over years and is known to cause late complications. Good metabolic control, i.e. lowering the % HbA1c, has proven to delay the onset and slowing the progression of diabetes late complications[3,4,5].

Principle of the assay

Afinion HbA1c Dx is a fully automated boronate affinity assay for the determination of the percentage of hemoglobin A1c in human whole blood.

The Afinion HbA1c Dx Test Cartridge contains all the reagents necessary for the determination of % HbA1c. The sample material is collected with the integrated sampling device before the test cartridge is placed in the cartridge chamber of the Afinion Analyzer. The blood sample is then automatically diluted and mixed with a solution that releases hemoglobin from the erythrocytes. The hemoglobin precipitates. This sample mixture is transferred to a blue boronic acid conjugate, which binds to the cis-diols of glycated hemoglobin. This reaction mixture is soaked through a filter membrane and all precipitated hemoglobin, conjugate-bound and unbound (i.e. glycated and non-glycated hemoglobin) remains on the membrane. Any excess of conjugate is removed with a washing reagent.

The analyzer evaluates the precipitate on the membrane. By measuring the reflectance, the blue (glycated hemoglobin) and the red (total hemoglobin) color intensities are evaluated, the ratio between them being proportional to the percentage of HbA1c in the sample. The % HbA1c is displayed on the Afinion Analyzer.

Standardization

Afinion HbA1c Dx is traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Reference Method for Measurement of HbA1c[6,7,8]. HbA1c values are reported according to the NGSP recommendations at DCCT (Diabetes Control and Complications Trial) level[3,7].

Afinion HbA1c Dx is certified by NGSP.

Materials provided (contents per 15 tests unit)

15 Test cartridges packed separately in foil pouches with a desiccant bag
1 Package insert

Materials required, but not provided with the kit

- Alere Afinion AS100 Analyzer (REF 1115175/1115390) or Afinion 2 Analyzer (REF 1116554/1116663/1116970/1116971)
- Afinion HbA1c Control (REF 1116975)
- Standard blood collection equipment
- Afinion User Manual (provided with the analyzer)
- Afinion HbA1c Dx Quick Guide (provided with the analyzer)

Electronic copies of Afinion User Manuals and Quick Guides are available at www.globalpointofcare.abbott

Description of the test cartridge

The main components of the test cartridge are the sampling device (1) and the reaction container (3). The test cartridge has a handle (4), a barcode label with lot specific information (5) and an ID area for sample ID (7). See figure and table below.

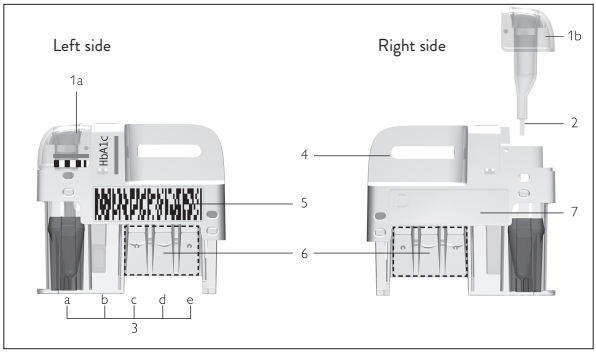


Figure 1 Afinion HbA1c Dx Test Cartridge.

Component		Function/composition
1	Sampling device a. Closed position b. Lifted position	For collection of patient sample or control.
2	Capillary	1.5 µL capillary to be filled with sample material.
3	Reaction container	Contains reagents necessary for one test:
	a. Conjugate	Blue boronic acid conjugate.
	b. Membrane tube	Tube with a polyethersulfone membrane.
	c. Washing solution	Morpholine buffered sodium chloride solution with detergents and preservative.
	d. Reconstitution reagent	HEPES buffered sodium chloride with lysis and precipitation agents.
	e. Empty	N/A
4	Handle	For correct finger grip.
5	Barcode label	Contains assay- and lot-specific information for the analyzer.
6	Optical reading area	Area for transmission measurement.
7	ID area	Space for written or labeled sample identification.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use test cartridges after the expiration date.
- Do not use test cartridges that have not been stored in accordance with recommendations.
- Do not use the test cartridge if the foil pouch is damaged.
- Do not use if the test cartridge itself has been damaged.
- Each foil pouch contains a desiccant bag with 1 g silica gel. This material shall not be used in the assay. Discard the desiccant bag in a suitable container. Do not swallow.
- Do not use the test cartridge if the desiccant bag is damaged and desiccant particles are found on the test cartridge. Do not wipe off.
- Do not touch the test cartridge optical reading area (figure 1).
- Do not reuse any part of the test cartridge.
- The test cartridge contains sodium azide (< 0.1 %) as a preservative. In case of leakage from the test cartridge, avoid contact with eyes and skin.
- The used test cartridges, sampling equipment, patient samples and controls are potentially infectious. Dispose immediately after use. Proper handling and disposal methods should be followed in accordance with local, state and federal regulations. Please also refer to the Safety Data Sheet available at https://www.globalpointofcare.abbott.
- Use personal protective equipment.

STORAGE AND STABILITY

Refrigerated storage 2-8°C (36-46°F)

- The Afinion HbA1c Dx Test Cartridges are stable until the expiration date only when stored refrigerated. The expiration date is stated on each foil pouch and on the kit box.
- The Afinion HbA1c Dx Test Cartridge must reach a temperature of 18-30°C (64-86°F) before use. Upon removal from the refrigerator, leave the test cartridge in the unopened foil pouch for at least 15 minutes. No test result will be obtained if the test cartridge is too cold when used. An information code will be displayed.
- Do not freeze.

Room temperature storage 15-25°C (59-77°F)

- The Afinion HbA1c Dx Test Cartridges can be stored in unopened foil pouches at room temperature for 90 days.
  Note the date placed at room temperature and the new expiration date on the kit box.
- Avoid exposure to direct sunlight.

Opened foil pouch

- The test cartridge should be used within 10 minutes after opening.
- Avoid exposure to direct sunlight.

SAMPLE MATERIALS AND STORAGE

The following sample materials can be used with the Afinion HbA1c Dx test:

- Capillary blood sample (from finger prick).
- Venous whole blood with anticoagulants: K_2-EDTA (EDTA=ethylene diamine tetra-acetic acid).

Sample storage

- Capillary blood samples cannot be stored.
- Venous whole blood with anticoagulants: K_2-EDTA, can be stored
  - refrigerated for 10 days
  - at room temperature (18-30°C, 64-86°F) for 8 hours.
- Do not freeze.
- Consult the Afinion HbA1c Control Package Insert for storage of control materials.

TEST PROCEDURE

Consult the Afinion HbA1c Dx Quick Guide for detailed instructions on how to collect and analyze a patient sample or control.

Test procedure overview

- Switch on the Afinion Analyzer.
- Allow the Afinion HbA1c Dx Test Cartridge to reach operating temperature 18-30°C (64-86°F). Open the foil pouch just before use.
- Be sure to properly label the test cartridge with sample ID. The test cartridge has a dedicated ID area.
- Collect a sample following the sample collection procedure described below. Once the capillary is filled, analysis of the test cartridge must start within 1 minute.
- Insert the test cartridge in the analyzer. The analysis time is approximately 3.5 minutes.

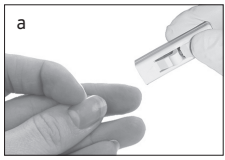
- Record the test results according to the laboratory guidelines. The results will be stored in the analyzer electronic result records.
- Remove the test cartridge from the analyzer.

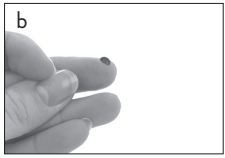
Important!
- Do not use test cartridges that have been accidentally dropped on the floor or lab bench after sample collection.
- Do not use cold test cartridges.
- Use the test cartridge within 10 minutes after opening the foil pouch.
- Analysis of the test cartridge must start within 1 minute after the capillary is filled with sample material.

Sample collection

Blood sampling from finger

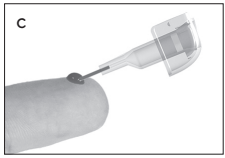
- Always use gloves.
- Clean the finger using alcohol. Allow the area to air dry.
- Use a lancet and firmly prick the finger (a). Properly dispose the lancet.
- Allow a good drop of blood to form before sampling (b).
- Apply direct pressure to the wound site with a clean gauze pad.





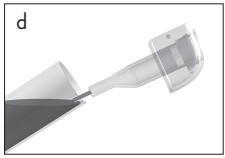
Sampling from a tube

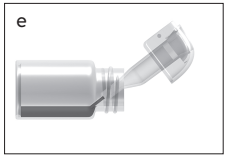
- Patient samples can be used directly from the refrigerator.
- Mix the sample material well. Invert the tube 8-10 times before collecting a sample.



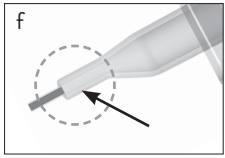
Sampling from the AFINION™ HbA1c Control vial

- Allow the control material to reach ambient operating temperature (18-30°C, 64-86°F) before use. This takes approximately 45 minutes.
- Mix the control material thoroughly by shaking the vial for 30 seconds. A Vortex mixer may be used.
- Extract a sample from the vial or the cap.





Important!
- Bring the tip of the capillary just beneath the surface of the blood drop/ sample material as shown in figures (c), (d) and (e).
- Be sure that the capillary is completely filled as shown in figure (f). It is not possible to overfill the capillary. Avoid air bubbles.
- Do not wipe off the capillary.



TEST RESULT REPORTING

Afinion HbA1c Dx measures the total glycated hemoglobin and the total hemoglobin concentration. The ratio between them is proportional to the % HbA1c of the sample. The analyzer calculates the ratio, and the test result is displayed as % HbA1c.

Reportable range

The Afinion HbA1c Dx measuring range is 4.00-15.00% HbA1c.
The HbA1c results are displayed in 0.01% intervals.
The hemoglobin measuring range is 6.0-20.0 g/dL.

Values outside the HbA1c measuring range

Valid for SW ≤ 7.03 (Afinion AS100) and SW ≤ 21.09 (Afinion 2):
If the patient's HbA1c value is outside the measuring range, no test result will be reported and an information code will be displayed (see "Troubleshooting").

Valid for SW ≥ 7.04 (Afinion AS100) and SW ≥ 21.10 (Afinion 2):

- HbA1c < 4.00 % is displayed if the measured HbA1c value is below 4.00%.
- HbA1c > 15.00 % is displayed if the measured HbA1c value is above 15.00%.

Expected values

The diagnostic cut-off is 6.5% HbA1c. Patients with HbA1c values in the range 5.7-6.4% are identified as having an increased risk for developing diabetes[2,9].

Interpretation of results

Despite a reliable internal process control of the analysis, each individual test result should be interpreted with careful consideration to the patient's medical history, clinical examinations and other laboratory results. If the test result is questionable or if clinical signs and symptoms appear inconsistent with the test result, analyze the Afinion HbA1c Controls and retest the sample using a new Afinion HbA1c Dx Test Cartridge.

Analytical specificity

Afinion HbA1c Dx measures the total glycated hemoglobin and reports the HbA1c value. Significant interference is defined as exceeding a 7% change in % HbA1c value from the reference.

Hemoglobin variants

Table 1: Sample profile for hemoglobin variant study.

Hemoglobin variant	Number of samples	Range of % content of variant	Range of concentration in %HbA1c
HbA0	100	82-95	4.3-13.9
HbA1a	100	0.4-1.4	
HbA1b	100	0.4-2.3	
HbA2	26	3.9-5.7	5.8-10.6
HbAS	21	35-42	5.6-8.8
HbAC	25	30-36	5.5-9.7
HbAE	20	17-26	6.1-8.9
HbAD	21	27-42	6.1-9.4
HbF	121	3.4-28.1	5.0-11.3

Table 2: Hemoglobin variant interference results. Percent relative bias from reference method at two levels of the HbA1c samples.

Hemoglobin Variant	Level 1: ~6.5 %HbA1c		Level 2: ~8.5 %HbA1c	
	Mean Relative bias (%)	Range %Bias^	Mean Relative bias (%)	Range %Bias^
HbA0	0.1	-6.1, 8.5	-0.4	-6.0, 6.7
HbA1a				
HbA1b				
HbA2	-3.4	-6.2, -1.3	-2.5	-6.6, 2.8
HbAS	-4.1	-6.6, 3.2	-1.0	-9.9, 3.5
HbAC	-5.5	-8.5, -1.8	-1.8	-5.2, 1.1
HbAE	3.5	1.4, 7.9	3.7	0.0, 4.7 (14.0*)
HbAD	-2.2	-4.1, 0.0	-2.9	-5.9, 0.0
HbF	10.4% HbF is the highest HbF concentration where no significant interference is observed with significant interference defined as > ±7%^^			

^ The range is defined as the minimum and maximum relative % difference at each concentration level.

^^ A negative relative bias with HbF has increasing magnitude with HbF and also with increasing %HbA1c.

* One single outlier for a sample with HbE level of 22.8%.

No significant interference was observed for the HbA2, HbAS, HbAC, HbAE and HbAD up to the levels stated. Significant negative interference was observed for individual samples with HbF concentrations above 10.4%. Please see section on Limitations of the test for further information.

Hemoglobin derivatives
No significant interference (< ±7%) was observed for samples with hemoglobin derivatives up to the following concentrations:

Acetylated Hb	4.6 mg/mL
Carbamylated Hb	13.8 mg/mL
Pre-glycated Hb	11.4 mg/mL

Limitations of the test

- This test should not be used to diagnose:
  - diabetes during pregnancy.
  - patients with an elevated fetal hemoglobin (HbF >10%) such as hereditary persistence of fetal hemoglobin (HPFH).
  - patients with a hemoglobinopathy but normal red cell turnover.
  - patients with abnormal red cell turnover (e.g., anemias from hemolysis and iron deficiency).
  - patients with iron deficiency and hemolytic anemia, various hemoglobinopathies, thalassemias, hereditary spherocytosis, malignancies, and severe chronic hepatic and renal disease.
  - patients that have received a blood transfusion within the past 3 weeks.
  - patients that have received cancer chemotherapy within the past 3 weeks.

- In cases of rapidly evolving type 1 diabetes the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- HbA1c testing should not replace glucose testing for type 1 diabetes, in pediatric patients and in pregnant women.
- Diluted samples cannot be used with Afinion HbA1c Dx.
- Coagulated or hemolyzed samples cannot be used with Afinion HbA1c Dx. Samples with hemolysis >14% (2000 mg/dL) may return an information code.
- If the sample has a hemoglobin value below 6.0 g/dL or above 20.0 g/dL, no test result will be reported and an information code will be displayed.

Interference

No significant interference (< ±7%) was observed up to the following concentrations:

- Bilirubin conjugated 600 mg/L
- Bilirubin unconjugated 600 mg/L
- Glucose 10 g/L
- Lipids (as Intralipid) 10 g/L
- Rheumatoid factor 780 000 IU/L
- Total protein 150 g/L
- Glycated albumin 7.7 g/L

Over-the-counter and prescription drugs:

- Acetaminophen 200 mg/L
- Acetylcysteine 1663 mg/L
- Acetylsalicylic acid 1000 mg/L
- Ampicillin 1000 mg/L
- Ascorbic acid 300 mg/L
- Cefoxitin 2500 mg/L
- Cyclosporine A 5 mg/L
- Cyclosporine C 5 mg/L
- Doxycycline 50 mg/L
- Glyburide 1.9 mg/L
- Heparin 5000 U/L
- Ibuprofen 500 mg/L
- Levodopa 20 mg/L
- Metformin 40 mg/L
- Methyldopa 20 mg/L
- Metronidazole 200 mg/L
- Phenylbutazone 400 mg/L
- Rifampicin 64 mg/L
- Salicylic acid 599 mg/L
- Theophylline 100 mg/L

- Hemolysis (in vitro) 14%
- Anticoagulant K_2-EDTA, at concentrations normally used in blood collection tubes, does not interfere.

Important!
It is possible that other substances and/or factors not listed above may interfere with the test and cause false results.

QUALITY CONTROL

Quality control testing should be done to confirm that your Afinion Analyzer System is working properly and providing reliable results. Only when controls are used routinely and the values are within acceptable ranges can accurate results be assured for patient samples.

Each laboratory site can benefit from establishing a quality control plan. The laboratory director should determine whether additional testing is appropriate for their laboratory.

It is recommended to keep a permanent record of all quality control results. The Afinion Analyzer automatically stores the control results in a separate record. Consult the Afinion Analyzer User Manual.

Control material

Afinion HbA1c Control is recommended for routine quality control testing. Consult the Afinion HbA1c Control Package Insert.

Frequency of control testing

Controls should be analyzed:

- with each new shipment of Afinion HbA1c Dx Test Kits.
- with each new lot of Afinion HbA1c Dx Test Kits.
- at least every 30 days.
- when training new operators.
- anytime an unexpected test result is obtained.

Make sure to test controls in compliance with any local, state and/or federal regulations.

Verifying the control results

The measured value should be within the acceptable limits for the control. Consult the Afinion HbA1c Control Package Insert.

If the measured value is outside the acceptable limits, make sure that:

- patient samples are not analyzed.
- the control vial is not expired.
- the control vial has not been in use for more than 60 days.
- the control vial has been stored according to recommendations.
- Afinion HbA1c Dx Test Cartridges have been stored according to recommendations.
- there is no visual sign of contamination of the control vial.

Correct any procedural error. Retest the control material.

If *no* procedural errors are detected:

- investigate the frequency of control failures.
- examine the laboratory's quality control records.
- ensure that there is no trend in out-of-range quality control results.
- retest the control material using a new control vial.
- patient results must be declared invalid. Contact customer service for advice. Do not analyze patient samples.

TROUBLESHOOTING

To ensure that correct HbA1c results are reported, the Afinion Analyzer performs optical, electronic and mechanical controls. The capillary, the test cartridge and all individual processing steps are checked during the course of each analysis. When problems are detected, the analyzer terminates the test and displays an information code.

The table below contains the Afinion HbA1c Dx assay specific information codes. Consult the Afinion Analyzer User Manual for information codes not listed in this table. Follow the actions listed in the user manual to correct the error.

Code #	Cause
103	The hemoglobin concentration is below 6.0 g/dL
104	The hemoglobin is above 20.0 g/dL
105	The HbA1c value is below 4.00%
106	The HbA1c value is above 15.00%

Important!
The manufacturer must be notified of any test system that is perceived or validated to be outside of the performance specifications outlined in the instructions.

Technical support
The manufacturer provides a toll free line for technical support.
Call 1-866-216-9505. The toll free number is available for use only in the United States of America.
E-mail: Afinion.Support@abbott.com

PERFORMANCE CHARACTERISTICS

The performance data presented in this section are representative data from internal and external studies. Results obtained in individual laboratories may vary.

Linearity

The linearity of the Afinion HbA1c Dx Assay was verified using two fresh EDTA blood samples. Varying amounts of sample 1 (17.9% HbA1c) and sample 9 (5.3% HbA1c) were mixed in different proportions to obtain a total of 9 samples. Sample 2-8 were analyzed in triplicate, while sample 1 and sample 9 (native samples) were analyzed in six replicates. A linear regression was calculated based on the theoretical vs. measured % HbA1c values. The results are shown in Table 3.

Table 3: Linear regression of Afinion HbA1c Dx. Measured (y) vs. theoretical (x) % HbA1c values. N=number of samples, r=correlation coefficient.

N	Regression line	r
9	y=1.01x + 0.07	1.00

The mean recovery of the measured % HbA1c values compared to the theoretical values (Table 4), were calculated for each sample, using the following equation:

$$\text{Mean recovery, (\%)} = \frac{\text{Measured mean value (\% HbA1c)}}{\text{Theoretical value (\% HbA1c)}} \times 100\%$$

Table 4: Linearity of Afinion HbA1c Dx. Theoretical and measured mean value (% HbA1c), Coefficient of Variation (CV) and recovery mean value.

Sample	Theoretical % HbA1c	Measured % HbA1c	CV (%)	Recovery (%)
1*	N/A	17.9	3.8	N/A
2	14.1	14.2	2.0	101
3	12.9	13.3	0.4	103
4	11.6	11.8	2.5	102
5	10.3	10.4	1.5	101
6	9.1	9.2	2.3	101
7	7.8	8.0	1.3	102
8	6.6	6.6	2.6	101
9*	N/A	5.3	2.1	N/A

*Native sample
N/A Not applicable

Method comparison

Fingerstick and venous whole blood samples from 120 patients (4.6-11.4% HbA1c), 38-42 at each of three sites, were analyzed using three different Afinion HbA1c Dx Lots. The venous samples were sent to a laboratory for duplicate analysis with an HPLC method. The results are shown in Table 5 and Table 6.

Table 5: Method comparison. Afinion HbA1c Dx vs. a laboratory HPLC method. Results from Weighted Deming and Passing-Bablok regression for 120 fingerstick samples (3 sites, 3 lots).

	Weighted Deming regression	Passing-Bablok regression
Slope	0.997	1.000
Intercept	0.000	-0.040
Correlation coefficient (r)	0.991	0.991
Number of samples	120	120
Results range Afinion (% HbA1c)	4.66 to 11.58	4.66 to 11.58

Table 6: Method comparison. Afinion HbA1c Dx vs. a laboratory HPLC method. Weighted Deming regression and Passing-Bablok slope and intercept for 120 venous whole blood samples (3 sites, 3 lots).

	Weighted Deming regression	Passing-Bablok regression
Slope	0.991	1.000
Intercept	0.053	-0.030
Correlation coefficient (r)	0.990	0.990
Number of samples	120	120
Results range Afinion (% HbA1c)	4.72 to 11.49	4.72 to 11.49

Precision

Within-laboratory precision – venous whole blood

Within-laboratory precision was determined according to CLSI Guideline EP05-A3. Four EDTA whole blood samples were analyzed for 20 days, 2 runs per day and 2 replicates per lot per run. 3 lots of Afinion HbA1c Dx and 3 analyzers were used. Precision data are summarized in Table 7.

Table 7: Within-laboratory precision estimates for whole blood samples. CV=Coefficient of Variation.

Sample	% HbA1c mean	%CV Repeat-ability	%CV Between-run	%CV Between-day
Low	5.14	1.18	0.00	0.60
Threshold	6.55	1.13	0.00	0.62
Medium	8.06	0.95	0.20	0.56
High	11.26	0.89	0.12	0.39

Sample	% HbA1c mean	%CV Between-lot	%CV Between-analyzer	%CV Total
Low	5.14	0.81	0.58	1.64
Threshold	6.55	0.85	0.00	1.51
Medium	8.06	0.47	0.49	1.31
High	11.26	0.88	0.52	1.42

Within-laboratory precision - controls

Within-laboratory precision was determined according to CLSI Protocol EP05-A3. Afinion HbA1c Control C I and Control C II were analyzed for 20 days, 2 runs per day and 2 replicates per run. 3 lots of Afinion HbA1c Dx and 3 analyzers were used. Precision data are summarized in Table 8.

Table 8: Within-laboratory precision estimates for Afinion HbA1c Controls. CV=Coefficient of Variation.

Sample	% HbA1c mean	%CV Repeat-ability	%CV Between-run	%CV Between-day
Control C I	6.35	0.86	0.38	0.25
Control C II	8.38	0.83	0.31	0.00

Sample	% HbA1c mean	%CV Between-lot	%CV Between-analyzer	%CV Total
Control C I	6.35	1.00	0.00	1.29
Control C II	8.38	0.41	0.28	1.01

External study – venous whole blood
The precision of the Afinion HbA1c Dx was assessed at three study sites. Each site tested four levels of HbA1c in venous whole blood samples (one sample at each level): low, medium, threshold and high. Three lots of Afinion HbA1c Dx were evaluated at each of the three study sites. Each sample was analyzed in 4 replicates with each lot, two times per day for 10 consecutive days, resulting in 240 determinations per sample. Within-run (repeatability), between-run, between-day and total %CV were calculated. Between-analyzer %CV from the internal study has been included in the total %CV. The results are shown in Table 9.

Table 9: Root-mean-squared coefficient of variation (CV) across the three study sites and three lots of Afinion HbA1c Dx.

Sample	% HbA1c mean	%CV Repeat-ability	%CV Between-run	%CV Between-day
Low	5.02	1.21	0.57	0.44
Threshold	6.41	1.11	0.48	0.40
Medium	8.18	1.10	0.23	0.28
High	12.09	0.97	0.38	0.25

Sample	% HbA1c mean	%CV Between-lot	%CV Between-analyzer	%CV Total
Low	5.02	0.92	0.58	1.78
Threshold	6.41	0.74	0.00	1.47
Medium	8.18	0.51	0.49	1.36
High	12.09	0.27	0.52	1.22

External study - controls
The precision of the Afinion HbA1c Control was assessed at three study sites. Each site tested the two levels of controls, Control C I and Control C II, with three lots Afinion HbA1c Dx. The controls were analyzed in 4 replicates with each lot, two times per day for 10 consecutive days. Within-run, between-run, between-day and total %CV was calculated. The results are shown in Table 10.

Table 10: Root-mean-squared coefficient of variation (CV) across the three study sites and three lots of Afinion HbA1c Dx.

Sample	% HbA1c mean	%CV Repeat-ability	%CV Between-run	%CV Between-day
Control C I	6.32	0.88	0.22	0.27
Control C II	8.48	0.79	0.02	0.26

Sample	% HbA1c mean	%CV Between-lot	%CV Between-analyzer	%CV Total
Control C I	6.32	0.39	0.31	1.08
Control C II	8.48	0.17	0.32	0.91

Precision – fingerstick samples
Precision components for fingerstick samples was estimated by combining data from three studies: Repeatability and between-lot variation were estimated from duplicate measurement of fingerstick samples from 172 subjects using 3 lots of test cartridges. Between-run and between-day components of variance were estimated from the within-laboratory precision study with venous whole blood. Between-analyzer and between-operator precision components were estimated from a study measuring four levels of HbA1c at three sites, with 5 or 6 subjects per level per site tested by three operators and two analyzers per site. This resulted in a total of 6 replicates per subject, and 15 or 16 subjects per level. The results are summarized in Table 11.

Table 11: Precision estimates for fingerstick samples. CV=Coefficient of Variation.

Sample	%CV Repeat-ability	%CV Between-lot	%CV Between-run	%CV Between-day
Low	1.52	1.20	0.00	0.60
Threshold	1.36	0.42	0.00	0.62
Medium	1.35	0.00	0.20	0.56
High	1.14	0.07	0.12	0.39

Sample	%CV Between-analyzer	%CV Between-operator	%CV Total
Low	0.14	0.00	2.03
Threshold	0.30	0.00	1.58
Medium	0.00	0.21	1.48
High	0.47	0.00	1.30

Between-analyzer precision
Between-analyzer precision of the Afinion HbA1c Dx was evaluated for 4 whole blood samples on 14 Alere Afinion AS100 Analyzers. For each sample, 6 replicates were analyzed on each analyzer with one test cartridge lot. The results are shown in Table 12.

Table 12: Precision estimates for whole blood samples. CV=Coefficient of variation.

Sample	% HbA1c mean	%CV Between-analyzer	%CV Total
Low	5.46	0.73	1.42
Threshold	5.47	0.87	1.33
Medium	6.54	0.82	1.37
High	8.85	0.99	1.36

Lot-to-lot variation

Lot-to-lot variation was evaluated with 18 whole blood samples and 3 lots of Afinion HbA1c Dx. Each sample was tested in one replicate with each of 3 lots of Afinion HbA1c Dx, using one Alere Afinion AS100 Analyzer for all three lots. The results are shown in Table 13.

Table 13: Bias, 95% confidence interval and 95% limits of agreement calculated for three lots of Afinion HbA1c Dx using the Bland-Altman analysis.

Comparison	Relative bias (%)	95% confidence interval of relative bias (%)	95% limit of agreement
Lot 3 – Lot 1	0.08	-0.8 to 1.0	-3.5 to 3.7
Lot 2 – Lot 1	-0.17	-1.1 to 0.8	-4.0 to 3.6
Lot 3 – Lot 2	0.25	-0.9 to 1.4	-4.4 to 4.9

Total error

Total error (TE) was calculated using the %bias estimates in the external method comparison study and the precision estimates in the external precision study. %TE is computed according to the following formula:

$$\%TE = |\%Bias| + 1.96 \times \%CV \times \left(1 + \frac{\%Bias}{100}\right)$$

Total error was calculated for both fingerstick and venous samples at four % HbA1c levels. The results are shown in Tables 14-16.

Table 14: %Total error (%TE) for fingerstick samples.

% HbA1c level	%CV	Weighted Deming regression		Passing-Bablok regression	
		%Bias	%TE	%Bias	%TE
5.0	2.03	-0.34	4.30	-0.80	4.75
6.5	1.58	-0.33	3.42	-0.62	3.69
8.0	1.49	-0.33	3.24	-0.50	3.41
12.0	1.30	-0.33	2.87	-0.33	2.87

Table 15: %Total error (%TE) for venous whole blood samples.

% HbA1c level	%CV	Weighted Deming regression		Passing-Bablok regression	
		%Bias	%TE	%Bias	%TE
5.0	1.64	-0.20	3.42	-0.60	3.80
6.5	1.51	-0.05	3.01	-0.46	3.41
8.0	1.31	-0.21	2.77	-0.38	2.93
12.0	1.42	-0.43	3.20	-0.25	3.03

Table 16: %Total error (%TE) for venous whole blood samples at the point of care.

% HbA1c level	%CV	Weighted Deming regression		Passing-Bablok regression	
		%Bias	%TE	%Bias	%TE
5.0	1.78	-0.20	3.69	-0.60	4.07
6.5	1.47	-0.05	2.93	-0.46	3.33
8.0	1.36	-0.21	2.87	-0.38	3.03
12.0	1.22	-0.43	2.81	-0.25	2.64

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SYMBOLS

The following symbols are used in the packaging material for Afinion HbA1c Dx.

CE	Conformity to the European directive 98/79/EC on *in vitro* diagnostic medical devices
IVD	*In vitro* diagnostic medical device
REF	Catalog number
LOT	Lot number
TEST CARTRIDGE	Test cartridge
Σ 1	Contents are sufficient for one test
Σ 15	Contents sufficient for 15 tests
	Do not reuse
	Consult instructions for use
	Caution, consult instructions for use
	Expiration date (year-month-day)
2°C 36°F 8°C 46°F	Storage temperature (store at 2-8°C, 36-46°F)
	Manufacturer
	Date of manufacture
℞ Only	Federal law restricts this device to sale by or on the order of a licensed healthcare professional

Abbott Diagnostics Technologies AS
Kjelsåsveien 161
P.O. Box 6863 Rodeløkka
NO-0504 Oslo, Norway
http://www.globalpointofcare.abbott

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