Title: Validation Plan Alere Afinion 2, HbA1C Dx	**Document ID:**
Department: General Laboratory	**Supersedes: N/A**
Author:	**Version Date:**
Approved By: Laboratory Director / Designee	**Implementation Date:**
Signature:	**Date:**

ANNUAL REVIEW (if necessary)

Signature	Date

Retirement of Policy and Procedure

Signature	Date

REVISION HISTORY

Version	Summary of Change
Pro 3000-01	Initial release.

OBJECTIVE:

Purpose of the Validation:

To demonstrate that the laboratory obtained the performance specifications comparable those established by the manufacturer for the following performance characteristics required by CLIA for moderate complex or unmodified tests: e.g.,

(A) Accuracy.
(B) Precision.
(C) Reportable range of test results for the test system.
(ii) Verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population.

Validation Plan:

The laboratory will follow its Method Validation Policy GEN 2260-1.

The laboratory will perform a Precision experiment.

The laboratory will perform Accuracy experiment.

The laboratory will perform a Reportable Range experiment.

The laboratory director will verify that the manufacturer's reference intervals (normal values) are appropriate for the laboratory's patient population, until the laboratory has enough patient data to establish normal population ranges.

Validation Documentation will Include:

Precision experiment
Reportable Range experiment
Correlation experiment
Correlation Source Documents Reference System
Correlation Source Documents New System
Proficiency Testing Enrollment Evidence
Testing Person Training and Competency Records
Test Report

BACKGROUND:

The Alere Afinion 2 HbA1c Dx is categorized as moderate complexity under the Clinical Laboratory Improvement Amendment of 1988 (CLIA'88).

PRODUCT DESCRIPTION

Intended use

The Afinion 2 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 laboratory will be validating its Afinion 2, serial number AF20032416 instrument

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 glycohemoglobin1.

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 diabetes2.

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 complications3,4,5.

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) level3,7.

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

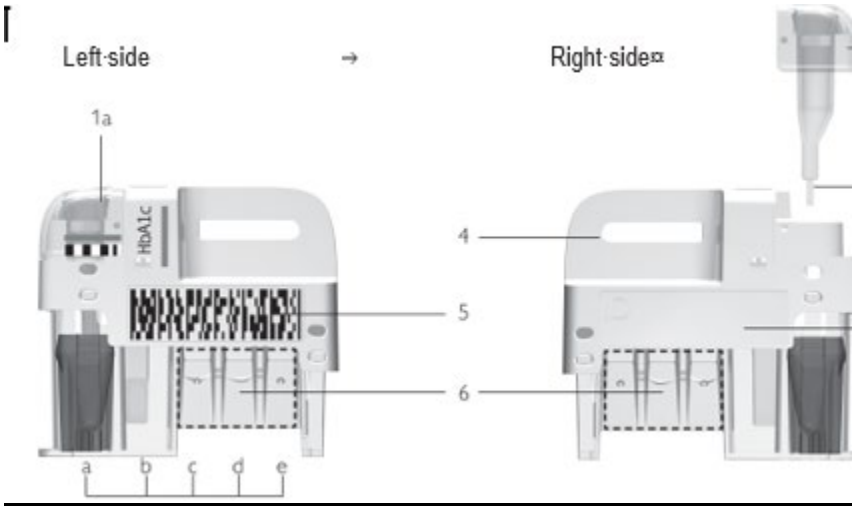
- Afinion 2 Analyzer
- Afinion HbA1c Control (1116975)
- Standard blood collection equipment)
- Afinion User Manual (provided with the analyzer)
- Afinion HbA1c Dx Quick Guide (provided with the analyzer)

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.



Component →	Function/composition
1. Sampling device →	For collection of patient sample or control.
a. Closed position	
b. Lifted position	
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 (59-77ºF)

- 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: K2-EDTA (EDTA=ethylene diamine tetra-acetic acid).

Sample storage

- Capillary blood samples cannot be stored.
- Venous whole blood with anticoagulants: K2-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.

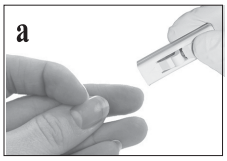
Important !

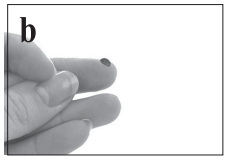
- **Do not** use test cartridges that have been accidentally dropped on the floor or lab bench after sample collection.
- **Do not** use cold test cartridges.
- Use the test cartridge within 10 minutes after opening the foil pouch.
- Analysis of the test cartridge must start within 1 minute after the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



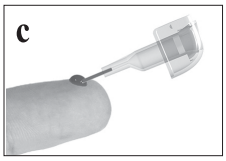


Sampling from a tube

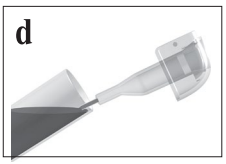
- Patient samples can be used directly from the refrigerator.
- Mix the sample material well. Invert the tube 8-10 times before collecting a sample.

Sampling from the AFINION™ HbA1c Control vial

- Allow the control material to reach ambient operating temperature (18-30ºC, 64-86ºF) before use.
  This takes approximately 45 minutes.

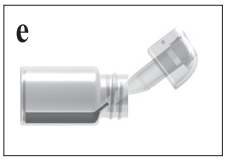


- Mix the control material thoroughly by shaking the vial for 30 seconds. A Vortex mixer may be used.
- Extract a sample from the vial or the cap.

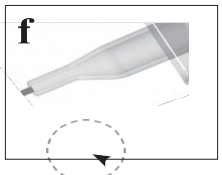


Important!

- 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.

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 bia from reference method at two levels of the HbA1c samples.

Hemoglobin Variant	Level 1: ~6. %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 an also with increasing %HbA1c.

* One single outlier for a sample with HbF 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%.

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.

QUALITY CONTROL

Quality control testing should be done to confirm that that 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

The laboratory will run 2 levels of control on each day of testing.

Control material

Afinion HbA1c or other appropriate control will be used for routine quality control testing.

1. **REFERENCE:** Medicare, Medicaid, and Clinical Laboratory Improvement Amendments (CLIA) Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications.
2. NCCLS. Clinical Laboratory Technical Procedures Manuals-Fourth Edition; Approved Guideline. (GP2-A4, 2002).
3. Sapient Bioanalytics Method Validation Policy – GEN 2260-01
4. Abbott Afinion HbA1c – Dx (Revision A 1117105, 2020/10) Instruction for Use (IFU)

Acknowledgment of Policy/Procedures

I have read and agree to follow the items mentioned in this validation plan.

Printed Name	Signature	Dept.	Date