GB Alere Afinion™ HbA1c

For use with the Alere Afinion™ AS100 Analyzer.

PRODUCT DESCRIPTION

Intended use

Alere Afinion™ HbA1c is an *in vitro* diagnostic test for quantitative determination of glycated hemoglobin (hemoglobin A1c, HbA1c) in human whole blood. The measure of HbA1c is recommended as a marker of long-term metabolic control in persons with diabetes mellitus[1]. This test can 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[2].

EC Certified (CE 0120) for self-testing: Alere Afinion™ HbA1c can be used for self-testing by lay users under professional supervision in a pharmacy. This test is not intended for use in a home environment.

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[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[3]. Good metabolic control, i.e. lowering the HbA1c concentration, has proven to delay the onset and slow the progression of diabetes late complications[4,5].

An International Expert Committee has concluded that measurements of HbA1c can be used to diagnose diabetes mellitus[2]. When in agreement with national regulations, Alere Afinion™ HbA1c can 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.

Principle of the assay

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

The Alere Afinion™ HbA1c Test Cartridge contains all the reagents necessary for the determination of the HbA1c concentration. The sample material is collected using the integrated sampling device and the Test Cartridge is placed in the Alere Afinion™ AS100 Analyzer. The blood sample is then automatically diluted and mixed with a liquid 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 Alere Afinion™ AS100 Analyzer evaluates the precipitate on the membrane. By measuring the reflectance, the blue (glycated hemoglobin) and the red (total hemoglobin) colour intensities are evaluated, the ratio between them being proportional to the percentage of HbA1c in the sample. The HbA1c concentration is displayed on the Alere Afinion™ AS100 Analyzer in the units mmol/mol, percentage (%), as the calculated estimated average glucose (eAG) or in combinations of these.

Kit contents (per 15 tests unit)

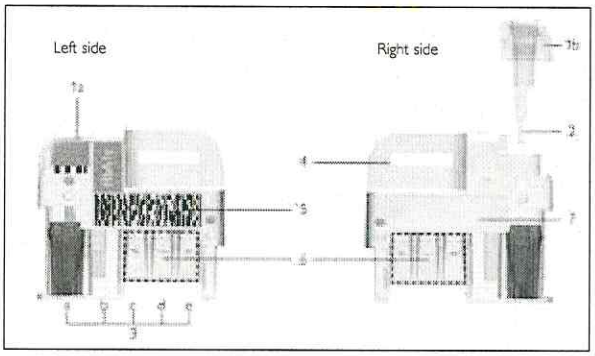
- 15 Test Cartridges packaged separately in foil pouches with a desiccant bag
- 1 Package Insert

Materials required but not provided with the kit

- Alere Afinion™ AS100 Analyzer
- Alere Afinion™ HbA1c Control
- Standard blood collection equipment

Description of the Test Cartridge

The main components of the Test Cartridge are the sampling device and the reagent container. The Test Cartridge has a handle, a barcode label with lot-specific information and an area for sample ID. See figure and table below.



Component	Function/composition
1 Sampling device a. Closed position b. Lifted position	For collection of patient sample or control.
2 Capillary	1.5 µL glass capillary to be filled with sample material.
3 Reaction wells a. Conjugate b. Membrane tube c. Washing solution d. Reconstitution reagent e. Empty	Contains reagents necessary for one test: Blue boronic acid conjugate. Tube with a polyethersulfone membrane. Morpholine buffered sodium chloride with detergents and preservative. HEPES buffered sodium chloride with lysis and precipitation agents. 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 labelled sample identification.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use this test to diagnose diabetes during pregnancy. It reflects the average blood glucose levels over the preceding 3 months (the average life of an erythrocyte), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival[2].

- Do not use Test Cartridges after the expiry date or if the Test Cartridges have not been stored in accordance with recommendations.
- Do not use the Test Cartridge if the foil pouch or the Test Cartridge 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.
- The Alere Afinion™ HbA1c Test Cartridge contains sodium azide as a preservative. The concentration is < 0.1%, which is below that which is considered hazardous in normal use[6]. In case of leakage, avoid contact with eyes and skin. Wash with plenty of water.
- Do not re-use any part of the Test Cartridge.
- The used Test Cartridges, sampling equipment, patient samples and controls are potentially infectious. The Test Cartridges should be disposed of immediately after use. Proper handling and disposal methods should be followed in accordance with local or national regulations. Use gloves.

STORAGE

Refrigerated storage (2-8°C)

- The Alere Afinion™ HbA1c Test Cartridges are stable until the expiry date only when stored refrigerated in sealed foil pouches. The expiry date is the last day of the month stated on the foil pouch and kit container.
- Do not freeze.

Room temperature storage (15-25°C)

- The Alere Afinion™ HbA1c Test Cartridges can be stored in un-opened foil pouches at room temperature for 90 days. Note the date of removal from the refrigerator on the kit container.
- Avoid exposure to direct sunlight.

Opened foil pouch

- The Test Cartridge must be used within 10 minutes after opening the foil pouch.
- Avoid exposure to direct sunlight.
- Avoid relative humidity above 90% (non-condensing).

SAMPLE MATERIAL

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

- Capillary blood (from finger prick)
- Venous whole blood with anticoagulants (EDTA, heparin, citrate or NaF)
- Alere Afinion™ HbA1c Control

Sample storage

- Capillary blood without anticoagulants cannot be stored.
- Venous whole blood with anticoagulants (EDTA, heparin or citrate) may be stored refrigerated for 10 days. Do not freeze.
- Consult the Alere Afinion™ HbA1c Control Package Insert for storage of control materials.

PREPARING FOR ANALYSIS

Consult the Alere Afinion™ AS100 Analyzer User Manual for detailed instructions on how to analyse a patient or control sample. The Alere Afinion™ HbA1c Quick Guide also provides an illustrated step-by-step procedure. The Alere Afinion™ HbA1c Quick Guide for Self Test provides an illustrated step-by-step procedure to be used by lay users in a pharmacy.

- The Alere Afinion™ HbA1c Test Cartridge must reach an operating temperature of 18-30°C before use. Upon removal from refrigerated storage, leave the Test Cartridge in unopened foil pouch for at least 15 minutes.
- Open the foil pouch just before use.
- Do not touch the Test Cartridge optical reading area.
- Label the Test Cartridge with patient or control ID. Use the dedicated ID area.

COLLECTING A SAMPLE

Capillary blood

- Make sure that the finger is clean, warm and dry.
- Use a suitable lancet to prick the finger.
- Allow a good drop of blood to form before sampling.

Venous blood

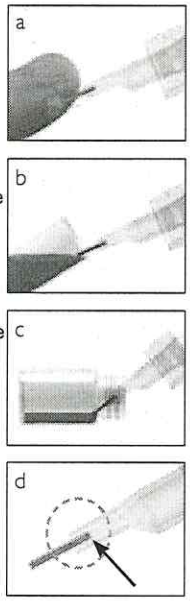
- Patient samples stored refrigerated can be used without equilibration to room temperature.
- Mix the sample material well by inverting the tube 8-10 times before collecting a sample.

Alere Afinion™ HbA1c Control

- Allow the control material to reach room temperature (15-25°C) before use, which takes approximately 45 minutes.
- Mix the control material thoroughly by shaking the vial for 30 seconds.
- The sample can be extracted from the vial or the cap.

Filling the capillary

- Remove the sampling device from the Test Cartridge.
- Fill the capillary; bring the tip of the capillary just beneath the surface of the patient sample (a, b) or control material (c). Be sure that the capillary is completely filled, see arrow (d). It is not possible to overfill. Avoid air bubbles and excess sample on the outside of the capillary. Do not wipe off the capillary.
- Immediately replace the sampling device into the Test Cartridge.
- The analysis of the Test Cartridge should start within 2-3 minutes. If the Test Cartridge is stored too long before analysis, the sample material may dry or coagulate. An information code will then be displayed. Consult the Alere Afinion™ AS100 Analyzer User Manual.
- Do not use a Test Cartridge that has been accidentally dropped on the floor or lab bench after sample collection.



ANALYSING A SAMPLE

- Analyse the Test Cartridge following the procedure described in the Alere Afinion™ AS100 Analyzer User Manual.
- The analysis time is 3 minutes and 15 seconds.

INTERPRETATION OF RESULTS

Interpret the Alere Afinion™ HbA1c test results 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, re-test the sample or confirm the result using another method. Analyse control materials frequently to verify the performance of the Alere Afinion™ AS100 Analyzer System.

Self-test use

The test result obtained should be interpreted with careful consideration to the patient's medical history and in collaboration with a health care provider. Lay users should not make any decision of medical relevance without consulting a health care provider.

HbA1c measuring units

Three different measuring units are in use for reporting HbA1c test results[8]:

- **mmol/mol** – the HbA1c values are aligned to the IFCC Reference Method for Measurements of HbA1c[9]
- **percentage (%)** – the HbA1c values are aligned to the assay used in the DCCT study, also known as NGSP-HbA1c[3,10,11]
- **estimated average glucose (eAG)** – the HbA1c value is converted to an equivalent average glucose value[12]

Linear relationships have been established between the three units[10,12]:

NGSP-HbA1c (%) = DCCT-HbA1c (%) = 0.092 x IFCC-HbA1c (mmol/mol) + 2.15
eAG (mmol/L) = 1.59 x DCCT-HbA1c (%) – 2.59

Measuring range

The Alere Afinion™ AS100 Analyzer displays the HbA1c value/ concentration in mmol/mol, percentage (%), as the calculated estimated average glucose (eAG) or in combinations of these:

	HbA1c	HbA1c	eAG
Unit	mmol/mol	%	mmol/L
Measuring range	20.0-140.0	4.0-15.0	3.8-21.3

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").

Important! Patient's HbA1c values should be reported in units consistent with the national recommendations. Please contact your local supplier if the national recommendation is unknown. Consult the Alere Afinion™ AS100 Analyzer User Manual for instructions on how to change the HbA1c measuring unit.

Standardisation

Alere Afinion™ HbA1c is traceable to the IFCC Reference Method for Measurement of HbA1c[5].

Expected values

Patients with HbA1c levels between 5.7 and 6.4 % (39 and 47 mmol/mol) are identified as being at risk for developing diabetes. The diagnostic cut-off is 6.5 % (48 mmol/mol)[2].

Analytical specificity

Alere Afinion™ HbA1c measures the total glycated hemoglobin and reports the HbA1c value. The following hemoglobin (Hb) variants have been analysed and found not to affect the Alere Afinion™ HbA1c test result: HbAC, HbAD, HbAE, HbF, HbAJ and HbAS[7]. Carbamylated and pre-glycated hemoglobin does not affect the Alere Afinion™ HbA1c test result.

Interference

No significant interference (< 5 %) was observed up to the following concentrations:

• Bilirubin	342 µmol/L (20 mg/dL)
• Triglycerides	15.7 mmol/L (1389 mg/dL)
• Cholesterol	9.1 mmol/L (351 mg/dL)
• Glucose	27.8 mmol/L (500 mg/dL)
• Fructosamine	680 µmol/L

Over-the-counter and prescription drugs:

• Acetaminophen	1.7 mmol/L (256 µg/mL)
• Ibuprofen	1.8 mmol/L (372 µg/mL)
• Acetylsalicylic acid	3.3 mmol/L (599 µg/mL)
• Salicylic acid	4.3 mmol/L (593 µg/mL)
• Glyburide	3.9 µmol/L (256 µg/mL)
• Metformin	310 µmol/L
• Hemolysis (*in vitro*)	5.0%

- Anticoagulants (EDTA, heparin, citrate and NaF) at concentrations normally used in blood collection tubes do not interfere.

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

Limitations of the test

- This test should not be used to diagnose[2]:
  - patients with a hemoglobinopathy but normal red cell turnover (e.g. sickle cell trait)
  - 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
- Diluted samples cannot be used with Alere Afinion™ HbA1c.
- Coagulated or hemolysed samples cannot be used with Alere Afinion™ HbA1c.
- If the sample has a hemoglobin value below 6 g/dL or above 20 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 your Alere Afinion™ AS100 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.

It is recommended to keep a permanent record of all quality control results. The Alere Afinion™ AS100 Analyzer automatically stores the control results in a separate log. Consult the Alere Afinion™ AS100 Analyzer User Manual.

Self-test use

Quality control is the responsibility of the pharmacy. Lay users are not required to run controls.

Choosing control material

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

If controls from another supplier are used, the precision must be determined and acceptable ranges for the Alere Afinion™ AS100 Analyzer System must be established. Lyophilised cell free lysate or hemolysed whole blood control materials cannot be used with Alere Afinion™ HbA1c.

Frequency of control testing

Controls should be analysed:

- Anytime an unexpected test result is obtained.
- With each shipment of Alere Afinion™ HbA1c test kits.
- With each new lot of Alere Afinion™ HbA1c test kits.
- When training new operators in correct use of the Alere Afinion™ HbA1c and the Alere Afinion™ AS100 Analyzer.
- In compliance with national or local regulations.

Verifying the control results

The measured value should be within the acceptable limits stated for the control material. Consult the Alere Afinion™ HbA1c Control package insert.

If the result obtained for the Alere Afinion™ HbA1c Control is outside the acceptable limits, make sure that:

- patient samples are not analysed until control results are within acceptable limits.
- the control vial has not passed its expiry date.
- the control vial has not been used for more than 60 days.
- the control vial and Alere Afinion™ HbA1c Test Cartridge have been stored according to recommendations.
- there is no evidence of bacterial or fungal contamination of the control vial.

Correct any procedural error and re-test the control material.

If no procedural errors are detected:

- Examine the laboratory's quality control record to investigate the frequency of control failures.
- Ensure that there is no trend in out-of-range quality control results.
- Re-test the control material using a new control vial.
- Patient results must be declared invalid when controls do not perform as expected. Contact your local supplier for advice before analysing patient samples.

TROUBLESHOOTING

To ensure that correct HbA1c results are reported, the Alere Afinion™ AS100 Analyzer performs optical, electronic and mechanical controls of the capillary, the Test Cartridge and all individual processing steps during the course of each analysis. When problems are detected by the built-in failsafe mechanisms, the Analyzer terminates the test and displays an information code.

The table below contains Alere Afinion™ HbA1c specific information codes. Consult the Alere Afinion™ AS100 Analyzer User Manual for information codes not listed in this table.

Code #	Cause
103	The hemoglobin concentration is below 6 g/dL
104	The hemoglobin concentration is above 20 g/dL
105	The HbA1c value is below measuring range
106	The HbA1c value is above measuring range

PERFORMANCE CHARACTERISTICS

Method comparison

A method comparison study, comprising 39 blood samples (4.9-11.7% HbA1c) were analysed with an affinity HPLC system and Alere Afinion™ AS100 Analyzer. A linear regression analysis and a Bland-Altman analysis were performed. The results are shown in Table 1 and 2.

Table 1: Method comparison. Alere Afinion™ HbA1c (y) vs. an affinity HPLC system (x). Linear regression analysis data. N=number of samples, r=correlation coefficient.

N	Regression line	r
39	y = 0.96x + 0.33	0.99

Table 2: Method comparison. Alere Afinion™ HbA1c vs. an affinity HPLC system. Bland-Altman analysis. N=number of samples, CI=Confidence Interval.

N	Bias	95% CI
39	0.00% HbA1c	-0.31- 0.30% HbA1c

Precision

Within-run, between-day and total precision values were determined according to CLSI Protocol EP5-A. Alere Afinion™ HbA1c Control C I, Control C II, one EDTA sample assayed for 20 days and one EDTA sample assayed for 17 days. The samples were analysed in duplicate twice a day. Precision data are summarised in Table 3.

Table 3: Within-run, between-day and total precision. N=number of days, CV=Coefficient of Variation.

Sample	N	Mean % HbA1c	Within-run CV (%)	Between-day CV (%)	Total CV (%)
Control C I	20	6.5	0.9	0.6	1.4
Control C II	20	9.1	0.6	0.5	1.1
Patient 1	17*	5.6	0.9	0.2	1.1
Patient 2	20	10.0	0.7	0.0	1.1

* Based on 17 days of analysis due to hemolysis of the sample.