ARCHITECT

SYSTEM

en

HbA1c

REF 4P72

B4P720

ABBL343/R06

Read Highlighted Changes
Revised February 2013

HbA1c

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

2°C 8°C	Store at 2-8°C	REACTION VESSELS	Reaction Vessels
SN	Serial Number	REAGENT LOT	Reagent Lot
REF	List Number	REPLACEMENT CAPS	Replacement Caps
LOT	Lot Number	SAMPLE CUPS	Sample Cups
GTIN	Global Trade Item Number	WARNING: SENSITIZER	WARNING: May cause an allergic reaction.
	Expiration Date	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
	Consult instructions for use.	WARNING: EYE IRRITANT	WARNING: Causes serious eye irritation.
IVD	*In Vitro* Diagnostic Medical Device	PRODUCT OF UK	Product of United Kingdom
SEPTUM	Septum	INFORMATION FOR USA ONLY	Information needed for United States of America only
	Manufacturer	PRODUCED FOR ABBOTT BY	Produced for Abbott by
CONTROL NO.	Control Number		

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

NAME

ARCHITECT HbA1c

INTENDED USE

The ARCHITECT HbA1c assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of percent hemoglobin A_{1c} (HbA_{1c}) in human whole blood on the ARCHITECT *i* System.

Percent HbA_{1c} measurements are used for monitoring long-term glycemic control in diabetic patients.

SUMMARY AND EXPLANATION OF TEST

HbA_{1c} is formed by the reaction of glucose with the N-terminal amino group of the hemoglobin beta chain. The Diabetes Control and Complications Trial (DCCT) Research Group previously reported a relationship between percent HbA_{1c} and mean blood glucose levels during the preceding 2-3 months.[1] The DCCT study also demonstrated that long-term control of diabetes can prevent complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy.

Measurement of percent HbA_{1c} is the method of choice for monitoring therapy of diabetic patients.[2,3]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HbA1c assay is a 2-step pre-treatment immunoassay for the quantitative determination of percent hemoglobin A_{1c} (% HbA_{1c}) in human whole blood using CMIA technology, with flexible assay protocols, referred to as Chemiflex.

Sample is incubated with pre-treatment reagent to lyse the red blood cells. Pre-treated sample is then incubated with magnetic microparticles with a silica surface. Hemoglobin and HbA_{1c} in the sample bind to the silica surface of the microparticles. Following a wash cycle, anti-HbA_{1c} acridinium-labeled conjugate is added to create a reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs).

The hemoglobin and HbA_{1c} that are bound to the surface of the microparticles represent the total percentage present in the sample however, only the HbA_{1c} result is required to determine the % HbA_{1c} in the sample. A direct relationship exists between the amount of HbA_{1c} in the sample and the RLUs detected by the ARCHITECT *i* System optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests/500 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT HbA1c Reagent Kit (4P72)

- **MICROPARTICLES** 1 Bottle (6.5 mL/26.5 mL) Silica surface microparticles in acetate buffer with zinc chloride and surfactants. Concentration: 0.15% solids. Preservative: ProClin 300.
- **CONJUGATE** 1 Bottle (5.8 mL/25.8 mL) Anti-HbA_{1c} (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with surfactants. Minimum concentration: 0.1 µg/mL. Preservatives: Sarafloxacin and Nipasept.
- **PRE-TREATMENT** 1 Bottle (9.8 mL/50.0 mL) Pre-treatment reagent (lysis buffer) containing detergent. Preservative: sodium azide.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION**: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[4] Biosafety Level 2[5] or other appropriate biosafety practices[6,7] should be used for materials that contain or are suspected of containing infectious agents.
- The following warnings and precautions apply to the Microparticles:

WARNING:	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/ eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P363	Wash contaminated clothing before reuse.

This material and its container must be disposed of in a safe way.

- The following warnings and precautions apply to the Pre-treatment reagent:

WARNING:	Contains Triton X-100 and sodium azide.
H319	Causes serious eye irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/ eye protection.
Response	
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/ attention.

This material and its container must be disposed of in a safe way.

- Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.
- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT HbA1c Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

- The ARCHITECT HbA1c Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, the reagents are stable until the expiration date.

- The ARCHITECT HbA1c Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.

- **WARNING: The pre-treatment reagent is prone to foaming. The septum must be removed and the bottle must be inspected for bubbles prior to loading on the ARCHITECT *i* System.**

- **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.**

- For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT HbA1c assay file must be installed on the ARCHITECT *i* System from an ARCHITECT *i* System Assay CD-ROM prior to performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT HbA1c assay is % HbA_{1c}. An alternate result unit, mmol/mol, may be selected for reporting results by editing assay parameter "Result concentration units" to mmol/mol. **Note:** The ARCHITECT *i* System will report mmol/mol as "mM/mol."
  - NGSP (% HbA_{1c}) values can be converted to IFCC (mmol/mol) values using the following equation:[8] [NGSP x 10.93] - 23.50
  - IFCC (mmol/mol) values can be converted to NGSP (% HbA_{1c}) values using the following equation:[8] [IFCC x 0.09148] + 2.152

Specimen Types

- The specimen collection tubes listed below were verified for use with the ARCHITECT HbA1c assay.
  - Human whole blood collected in:
    - Dipotassium EDTA
    - Sodium Fluoride/Potassium EDTA
    - Fluoride Oxalate
    - Sodium Fluoride/Sodium EDTA
- Do not use lithium heparin specimen collection tubes with this assay.
- Other specimen collection tubes have not been verified for use with this assay.
- Performance has not been established for cadaveric specimens or for the use of body fluids other than human whole blood.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT HbA1c assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - obvious microbial contamination
- For accurate results, specimens should be free of clots and other particulate matter.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes.

Fresh/Non-Frozen Samples

- Do not centrifuge fresh/non-frozen samples.
- All fresh/non-frozen samples must be inverted or mixed immediately (*i.e.*, within 2 minutes) prior to loading onto the instrument.

Frozen Samples

- Samples that have been frozen for a minimum of 2 hours at -20°C or colder may be used without restriction in the ARCHITECT HbA1c assay when prepared as follows:
  - Frozen specimens must be completely thawed before mixing.
  - Mix thawed specimens thoroughly by inverting 10 times or by low speed vortexing. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous. If samples are not mixed thoroughly, inconsistent results may be obtained.
  - To ensure consistency in results, frozen and thawed specimens must be centrifuged at >10,000 RCF (Relative Centrifugal Force) for 5 minutes before testing.

Storage

- If testing will be delayed, whole blood specimens may be stored
  - up to 7 days at room temperature or
  - up to 14 days at 2-8°C.
- If testing will be delayed more than 14 days, store at -20°C or colder.[9]
- Avoid more than 1 freeze/thaw cycle.

Shipping

- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient, at 2-8°C (wet ice), or frozen (dry ice). Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 4P72 ARCHITECT HbA1c Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT HbA1c Assay file, may be obtained from:
  - ARCHITECT *i* System e-Assay CD-ROM found on www.abbottdiagnostics.com
  - ARCHITECT *i* System Assay CD-ROM
- 4P72-01 ARCHITECT HbA1c Calibrators
- Commercially available control material containing HbA_{1c}.
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the specified volumes.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT HbA1c Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Handling Precautions** section of this package insert.

- **WARNING: Visually inspect the pre-treatment reagent bottle for bubbles. Remove bubbles with an applicator stick before placing a septum on the bottle. Refer to the ARCHITECT System Operations Manual, Section 5. Alternatively, allow the pre-treatment reagent to stand for 30 minutes to allow the bubbles to dissipate.**

- Load the ARCHITECT HbA1c Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present before running the test.
  - Priority: 70 µL for the first ARCHITECT HbA1c test plus 20 µL for each additional test from the same sample cup.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
  - Refer to the **Preparation for Analysis** section of this insert for frozen/thawed samples.

Fresh/Non-Frozen Samples

Warning: The ARCHITECT HbA1c assay utilizes whole blood samples. There is a risk of red blood cell settling in fresh/non-frozen samples which may result in falsely low % HbA_{1c} values.

To minimize the effects of red blood cell settling, all of the following steps MUST be followed for fresh/non-frozen samples:

1. All fresh/non-frozen samples must be priority loaded.
2. Ensure that no other samples are in the priority bay prior to loading the HbA_{1c} samples.
3. All fresh/non-frozen samples must be inverted or mixed immediately (*i.e.*, within 2 minutes) prior to loading onto the instrument.
4. No more than 20 tests (for the ARCHITECT *i* 2000/*i* 2000_{SR}) and no more than 10 tests (for the ARCHITECT *i* 1000_{SR}) can be processed from primary tubes or sample cups at one time. Running more whole blood tests at one time will increase the risk of red blood cell settling during the run.
5. All fresh/non-frozen samples must be aspirated within 15 minutes of being mixed and placed on the ARCHITECT *i* System.

Frozen/Thawed Samples

Note: The process of freezing and thawing whole blood samples causes the red blood cells to lyse therefore frozen/thawed samples are not affected by red blood cell settling.

- ≤ 3 hours on-board for frozen and thawed samples: 150 µL for the first ARCHITECT HbA1c test plus 20 µL for each additional test from the same sample cup.
- > 3 hours on-board for frozen and thawed samples: replace with new thawed samples (patient specimens, controls, and calibrators).

- Prepare calibrators and controls.
  - ARCHITECT HbA1c Calibrators should be prepared according to the instructions in the ARCHITECT HbA1c Calibrator package insert.
  - To obtain the recommended volume requirements for the ARCHITECT HbA1c Calibrators, hold the bottles **vertically**, and dispense 3 drops of each calibrator into each respective sample cup.
  - Follow the manufacturer's instructions for preparation of commercially available control material.
- Load samples
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

- Specimens with HbA_{1c} concentrations greater than 14.5% HbA_{1c} will be flagged as "> 14.5 %HbA1c" and may be diluted using the Manual Dilution Procedure.

Manual Dilution Procedure

- The suggested dilution for the ARCHITECT HbA1c assay is 1:2.
- Add 250 µL of the patient specimen to 250 µL of a low HbA_{1c} sample (*i.e.*, < 6% HbA_{1c}) and mix thoroughly before testing.
  - Note: the manual dilution procedure is only valid where both the patient specimen and the low HbA_{1c} sample have Hb concentrations in the range of 7 to 20 g/dL.
- The result should be greater than 4.0% HbA_{1c} before the dilution calculation is applied.
- Calculate the value of the sample using the following equation
  - Sample Value (%) = (Observed Concentration x 2) - Low Sample Concentration
- **NOTE: Manual dilution factors cannot be entered into the Patient or Control order screen. However, for maintenance of detailed information (records), select *Patient Order* then select the appropriate assay. Select *Sample Details F2*. Enter the dilution factor in the Comment Box.**
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT HbA1c calibration, test calibrators A, B, C, D, E, and F in replicates of 2. The calibrators should be priority loaded.
- Calibration Range: 4.0 to 14.5% HbA_{1c}.
- To evaluate the calibration of this assay using commercially available controls, a single sample of each control level must be tested to evaluate the assay calibration.
  - Order controls as described in the **Assay Procedure** section.
  - Ensure that assay control values are within the established ranges.
- Once an ARCHITECT HbA1c calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

- The recommended control requirement for the ARCHITECT HbA1c assay is that a single sample of each control be tested once every 24 hours each day of use. If quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures or your federal, state, and/or local accrediting agency requirements or regulations.
- Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. In addition, each laboratory should establish its own concentration ranges for new control lots at each control level employed. These ranges should be established according to your laboratory quality control policy and/or any local, state, and/or federal regulations or accreditation requirements. Concentration ranges provided in the control package insert should be used only for guidance.
- For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.
- If a control is out of its established range, the associated test results may be invalid and should be retested per laboratory procedures. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT HbA1c assay belongs to method group 3.

RESULTS

- The ARCHITECT HbA1c assay uses a point to point data reduction method to generate a calibration curve.
- The ARCHITECT HbA1c assay results can be used to manually calculate the estimated Average Glucose (eAG) with the following equation:[10]
  - eAG (mg/dL) = (28.7 x % HbA_{1c}) - 46.7

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measurement Range (Reportable Range)

Measurement range is the range of values in % HbA_{1c} which meets the limits of acceptable performance for both imprecision and bias for an undiluted sample. The range is 4.0% HbA_{1c} to 14.5% HbA_{1c}.

LIMITATIONS OF THE PROCEDURE

- If the HbA_{1c} results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Results should be used in conjunction with other data; *e.g.*, symptoms, results of other tests, clinical impressions, etc.
- This assay is not intended for:
  - The diagnosis of diabetes mellitus.[1,2]
  - Monitoring daily glucose control and should not be used to replace daily home testing of urine and blood glucose levels.
  - Analyzing samples from patients with conditions causing shortened red blood cell survival, such as hemolytic diseases, pregnancy, significant acute or chronic blood loss.[11-15]
  - Analyzing samples from patients with total hemoglobin levels < 7 or > 21 g/dL (< 70 or > 210 g/L).
- Hemoglobinopathies may interfere with glycated hemoglobin (GHb) analysis.[16] Samples containing the following hemoglobin variants have been shown to interfere with the ARCHITECT HbA1c assay: Hemoglobin D, Hemoglobin E, Hemoglobin F (>9%), and Hemoglobin S. Refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS, Specificity** section for further explanation.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[17,18] Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT HbA1c that employ mouse monoclonal antibodies.[17]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[19] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Do not use lithium heparin specimen collection tubes with this assay.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

EXPECTED VALUES

For monitoring diabetic patients, it is recommended that glycemic goals are individualized following current professional society recommendations.[20]

The American Diabetes Association (ADA) recommendations[20] are summarized in the following table.

HbA_{1c} Value	**Glycemic Goal**
< 8% HbA_{1c} (64 mmol/mol)	Less Stringent
< 7% HbA_{1c} (53 mmol/mol)	General (Non-Pregnant Adults)
< 6.5% HbA_{1c} (48 mmol/mol)	More stringent

As recommended by the ADA, patients in the range of 5.7 - 6.4% HbA_{1c} (39 - 46 mmol/mol) would be in the category of increased risk for diabetes.[20]

SPECIFIC PERFORMANCE CHARACTERISTICS

Data in the **SPECIFIC PERFORMANCE CHARACTERISTICS** section were generated using the ARCHITECT *i* 2000_{SR} System.

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT HbA1c assay is designed to have an imprecision of ≤ 5.0% within-laboratory (Total) CV for samples with % HbA_{1c} values ranging from 4.0 to 14.5%.

A study was performed based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.[21] Testing was conducted using 2 lots of ARCHITECT HbA1c Reagents and Calibrators, 1 lot of commercially available controls (Bio-Rad Lyphochek Diabetes Controls), and 2 instruments. Three levels of human whole blood panels were assayed in a minimum of 2 replicates at 2 separate times per day for 20 different days. Each reagent lot used a stored calibration curve throughout the study. The data are summarized in the following table.

Sample	Instrument	Reagent Lot	n	Mean (% HbA_{1c})	Within-Run		Total	
					SD	%CV	SD	%CV
Panel 1	1	1	80	5.1	0.09	1.8	0.15	2.9
	2	2	80	5.1	0.14	2.8	0.18	3.6
Panel 2	1	1	80	6.3	0.15	2.4	0.19	3.0
	2	2	80	6.2	0.14	2.3	0.19	3.1
Panel 3	1	1	80	9.8	0.21	2.1	0.27	2.8
	2	2	80	9.9	0.28	2.8	0.38	3.8

Linearity

The ARCHITECT HbA1c assay is designed to be linear across the measurement range of 4.0 to 14.5% HbA_{1c}.

A study was performed based on guidance from the NCCLS document EP6-A.[22] Nine dilution series were prepared as follows: a high % HbA_{1c} sample was combined in specific ratios with a low % HbA_{1c} sample. The 9 dilution series, including the low-level and high-level samples, were tested using the ARCHITECT HbA1c assay. The ARCHITECT HbA1c assay demonstrated linearity from 4.0 to 15.8% HbA_{1c}.

Dilution Verification

The ARCHITECT HbA1c is designed to have a mean deviation in concentration of ≤ 10% when comparing the result from a manually diluted sample to the result of an undiluted (neat) sample.

The ARCHITECT HbA1c manual dilution method was evaluated using 3 human whole blood samples with % HbA_{1c} levels ranging from 15.9 to 17.1%, and 3 human whole blood samples with % HbA_{1c} levels ranging from 5.3 to 5.8%. To perform each dilution, 250 µL of high % HbA_{1c} sample was manually diluted 1:2 using 250 µL of low % HbA_{1c} sample to create a total of three manually diluted samples. The manually diluted samples were assayed in replicates of 3 on 1 instrument using the ARCHITECT HbA1c assay. The percent difference values were 7.2, 0.8, and -0.5 for the three samples.

Specificity

Effect of Total Hemoglobin Levels on Percent HbA_{1c}

The ARCHITECT HbA1c assay is designed to have a mean deviation in concentration of ≤ 10% between 6.0 and 8.0% HbA_{1c} for samples with total hemoglobin levels of approximately 7 and 21 g/dL when compared with hemoglobin (Hb) in the normal range.

The effect of varying amounts of total hemoglobin on the ARCHITECT HbA1c assay was assessed using three IFCC reference controls with a target value of 6.96% HbA_{1c}. The data are summarized in the following table.

Sample	Total Hemoglobin (g/dL)	Measured % HbA_{1c}	% Difference (% HbA_{1c})[a]
IFCC Normal Hb	13	6.9	-
IFCC Low Hb	7	6.3	-8.2 %
IFCC High Hb	20	7.0	1.3 %

[a] $$\% \text{ Difference} = \frac{\text{Mean Low or High Hb Sample - Mean Normal Hb Sample}}{\text{Normal Hb Sample}} \times 100$$

Hemoglobin Derivatives

The ARCHITECT HbA1c assay is designed to have a mean deviation in concentration of ≤ 10% in samples containing labile glycated hemoglobin (which has a Schiff base attachment of glucose to HbA).

High concentrations of glucose (1400 mg/dL) were spiked into 2 human whole blood samples with different levels of % HbA_{1c} (approximately 6.5 and > 8.0%) and incubated for 3 hours at 37°C to generate labile glycated hemoglobin. Mean percent differences of -1.1 and 0.8 were obtained when comparing samples with glucose levels of 1400 mg/dL to the reference samples with % HbA_{1c} values ranging from approximately 6.5 to > 8.0%.

Hemoglobin Variants

The hemoglobin variants listed in the table below were evaluated to determine the affect on % HbA_{1c} values when using the ARCHITECT HbA1c assay as compared to a reference assay (IFCC). The data are summarized in the following table.

Hemoglobin Variant	n	Mean % Interference from IFCC[a]	% Interference Range[a]
HBA2	12	-0.4	-5.9 to 5.6
HbC	12	2.0	-2.8 to 7.1
HbD	12	28.6	19.1 to 36.3
HbE	12	21.1	-24.1 to 90.4
HbF[b]	11	-2.4	-12.9 to 4.9
HbJ	12	2.5	-9.8 to 9.6
HbS	11	2.1	-7.8 to 10.5

[a] $$\% \text{ Interference} = \frac{\text{Observed Bias - Expected Bias}}{\text{IFCC \% } HbA_{1c} \text{ Value}} \times 100$$

[b] Two samples containing HbF at > 9% demonstrated interference. Refer to the **LIMITATIONS OF THE PROCEDURE** section of this package insert for further information.

Interference

The ARCHITECT HbA1c assay is designed to have a mean deviation in concentration of ≤ 10% when comparing spiked samples to reference samples.

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2.[23] Potential interferents were evaluated to determine whether % HbA_{1c} values were affected when using the ARCHITECT HbA1c assay. The potential interferents listed below were spiked into human whole blood samples with different levels of % HbA_{1c} (approximately 6.5 and > 8.0% HbA_{1c}). The samples were assayed, and the % HbA_{1c} values of the spiked samples were compared to reference samples. The data are summarized in the following table.

		% Interference[a]	
Potential Interferent	**High Test Level**	**6.5 % HbA_{1c}**	**> 8.0 % HbA_{1c}**
Bilirubin (total)	50 mg/dL	-3.7	-4.5
Total Protein	5 g/dL	-1.9	-5.6
Triglycerides	1600 mg/dL	-2.8	-3.5
Rheumatoid Factor	800 IU/mL	-1.0	-0.9
Acetylsalicylate	66 mg/dL	0.6	3.6
Ascorbic acid	50 mg/dL	5.3	2.7
Sodium Cyanate	50 mg/dL	-0.3	-2.9
Urea	667 mg/dL	2.1	0.5

[a] $\%\ \text{Interference} = \frac{\text{Test Result - Control Result}}{\text{Control Result}} \times 100$

Method Comparison

The ARCHITECT HbA1c assay is designed to have a slope of 1.0 ± 0.1 and a correlation coefficient (r) of ≥ 0.9 for specimens across the range of 4.0 to 14.5% HbA_{1c} when compared to a commercially available diagnostic kit.

A correlation study using human whole blood specimens (n = 127) was performed based on guidance from the NCCLS Document EP9-A2.[24] The specimens were evaluated using the Passing-Bablok regression method for the slope and the Pearson regression method for the correlation coefficient (r). The data are summarized in the following table.

Concentration Range (% HbA_{1c})		**Correlation Coefficient (r) (95% CI[a])**	**Slope (95% CI[a])**	**Intercept (95% CI[a])**
ARCHITECT	**Comparator**			
4.07 - 13.61	4.75 - 14.30	0.95 (0.93, 0.96)	1.04 (0.97, 1.12)	-0.07 (-0.67, 0.37)

[a] = CI = Confidence Interval

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