HbA_1c DIRECT
HbA_1c DIRECT TURBIDIMETRY En

REF 01R87-51 2286 Tests
REF 01R87-41 1143 Tests
REF 01R87-31 826 Tests
REF 01R87-21 571 Tests

Test for the quantitative turbidimetrically determination of HbA1c (%) human whole blood.
Liquid reagents. Store at 2°C - 8°C. Do not freeze. For in Vitro Diagnostic Use.

***** 01R87-51 / 01R87-41 / 01R87-21 / 01R87-31 Ref Number Products are Produced Specifically for Abbott Architect Biochemistry Analyzer Series

INTENDED USE

The Archem HbA1c assay is used in clinical laboratories for the quantitative in vitro measurement of HbA1c (hemoglobin fraction) in human whole blood on auto analyzers. The HbA1c assay is used as an aid in the monitoring of long-term blood glucose control and compliance in individuals with diabetes mellitus.

SUMMARY AND EXPLANATION OF TEST

The Archem HbA1c assay is used for the measurement of the concentration of HbA1c relative to the concentration of the total hemoglobin (THb).

Individuals diagnosed with diabetes mellitus have been found to have an increased percent HbA1c. Uncontrolled diabetes can lead to acute complications of hyperglycemia and ketosis. In addition, long-term complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy can occur. According to several studies, including the findings of diabetes Control and Complications Trial (DCCT), these complications can be prevented by long-term control of diabetes.

Therefore, measurement of percent HbA1c can be invaluable in the monitoring of long-term glycemic control of diabetic patients.

The Archem HbA1c assay has no cross reactivity with labile HbA1c since the antibody used in this assay is specific for the ketoamine form of HbA1c. The stable HbA1c provides a measure of the individual's blood mean glucose blood level for the previous several months because it does not fluctuate in response to rapid changes in physiological factors.

Correlation between HbA1c and DAYTIME Glucose levels		
HbA1c (%)	Gluc. (mg/dL)	Gluc. (mmol/L)
5	97	5,4
6	126	7
7	154	8,6
8	183	10,2
9	212	11,8
10	240	13,4
11	269	14,9
12	298	16,5
Values are sourced from NGSP global website 2013-2014		

TEST PRINCIPLE

Hemoglobin A1c is an important test recommended by the American Diabetes Association (ADA) and its usefulness was clarified by the United Kingdom Prospective Diabetes Study (UKPDS) and Diabetes Control and Complications Trial (DCCT). Currently, the HbA1c test is recommended for patients with diabetes every 2-3 months as part of the patient diabetes management program. Glycohemoglobin is produced by non-enzymatic addition of glucose to amino groups in hemoglobin. HbA1c refers to glucose modified hemoglobin A (HbA) specifically at N-terminal valine residues of hemoglobin beta chains. HbA1c test is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications. Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months.

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same

unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse anti human HbA1c antibody complex is formed. Agglutination is formed when goat antimouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

• Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.

TEST PARAMETERS

Method	: Immunoturbidimetric
Wavelength	: 660 nm
Temperature	: 2-8ºC
Sample	: Whole blood with EDTA
Linearity	: 4.5% - 16% (NGSP)

REAGENT COMPOSITION

Lyse Reagent	Reagent R1	Reagent R2:
Stabilizers Buffers, lysing agent, water	Latex: < 0,15 % Buffer Stabilizers.	Mouse anti-human HbA1c monoclonal antibody< 0.06mg/ml, goat anti-mouse IgG polyclonal antibody < 0.09mg/dl, Buffer, stabilizers.

Materials Required but not Provided

1) HbA1c calibrator set Intended for use only with Direct Turbidimetric HbA1c Assay reagents
2) Bi-level HbA1c controls Whole blood hemolysates and stabilizers. (Cat No: HBCN01 (01R96-01))

REAGENT PREPARATION

R1: Liquid, ready to use.
R2: Liquid
Lyse Reagent: Liquid, ready to use.

REAGENT STABILITY AND STORAGE

Reagents are stable until their expiration date when stored at 2-8°C.
Reconstituted R2ab thus prepared is stable for 1 month when stored at 2-8°C. **R1 and R2 reagents are light sensitive.** Once opened vials R1 and R2 are stable 30 days minimum at 2-8ºC. There is a strong relation between on board stability and analyzers specifications.

SPECIMEN COLLECTION AND HANDLING

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 2 weeks of collection when stored refrigerated. Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

Auto analyzer usage: Samples should be tested by stat mode (Emergency mode) for avoiding precipitation.

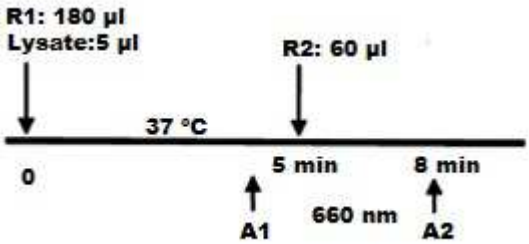
TEST PROCEDURE

Whole Blood Bench Top Lysis Procedure

1) Dispense 250 µL of Lysis reagent in a sample cup or an Eppendorf microfuge tube.
2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes. **Accuracy of the assay will be affected if whole blood is not mixed prior to testing.** Add 5 µL of fully resuspended whole blood sample to the lysis buffer in the sample cup or microfuge tube. Mix gently with a suitable pipettor without creating foam and incubate at room temperature (25°C) for 5-10 minutes to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter. Incubate the samples longer as needed to ensure complete hemolysate preparation. The lysate, thus prepared, is ready for use in the Direct Turbidimetric HbA1c assay steps and is stable up to 2 days at 2-8 °C. This process can be performed by Auto analyzers automatically.
3) The calibrators and controls should be treated exactly as patient samples and used per instructions on labeling.

ASSAY SCHEME FOR ANALYSERS

For analyzers capable of handling only 2-reagents, use the following scheme as a guideline for analyzer application. Note: HbA1c is an end-point assay and the first reading point A1 is right before the addition of reagent R2.



CALIBRATION

The Archem Direct Turbidimetric HbA1c assay requires monthly calibration. Place calibration series on the analyzer in the order of lowest to highest. Enter calibrator lot specific values provided on the specification sheet.

Archem Direct Turbidimetric HbA1c calibrator sets are intended for use with Hemoglobin A1c Turbidimetric assay reagents.

Cat No: HBCL04 (01R97-01)

All calibrator vials are stable until their expiration date when stored at 2-8°C. Archem HbA1c calibrator set is in lyophilized form. Archem HbA1c calibrator set for the auto analyzers on-board Lysis Application includes four levels of calibrator material. Levels 1-4 are in lyophilized form. Reconstitute lyophilized contents per instructions on labeling and mix gently. Let the vials equilibrate at room temperature for 30 minutes before use. Reconstituted calibrators are stable for 30 days when capped tightly and stored at 2-8°C.

*Calibration Stability: It is strongly depend of application to auto analyzers and auto analyzers specification. Calibration stability is 30 days in ARCHITECT c Systems.

QUALITY CONTROL

Archem Direct Turbidimetric HbA1c control set can be purchased separately. Users should follow the appropriate local guidelines concerning the running of external quality controls and handling of bio-hazardous material.

The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c. The values reported are aligned with the Diabetes Control and Clinical Trials (DCCT) system and hence reported in the NGSP% format. No calculation step is needed.

The International Federation of Clinical Chemistry (IFCC) values can be calculated by use of published10.11.
Conversion formula:
NGSP %= [0.0915 x (IFCC) mmol/mol] + 2.15.

REFERENCE INTERVALS (NORMAL VALUES)

Expected Values: %4.5 - 6.5 (NGSP/DCCT)
Expected Values: 26 - 48 mmol/mol (IFCC)
Expected Values: %4.5 - %7 (Diabetics) (NGSP/DCCT)

Recommended Values: less than 6.5% (This value had been set according NGSP regulation at 2013) for a non-diabetic, less than 7% for glycemic control of a person with diabetes and pregnants. That is, the patient should be monitored against him or herself. There is a 3-4 week time lag before Hemoglobin A1c reflects changes in blood glucose level. Hemoglobin A1c to monitor diabetic patients, results should be inter pretend individually.

Not only the findings of a single test result but also an integration of both clinical and laboratory data should be used in clinical diagnosis.

*Each laboratory should establish its own expected values.

Limitations

- The linearity of the assay is up to 16% HbA1c. Samples with values above 16% should not be diluted and retested. Instead the values should be reported as higher than 16% (>16%).
- It has been observed that patient who has alcoholism, high dose of acetyl salicylic acide, opiate and lead poisoning may lead to inconsistency.
- The assay is formulated for use with human whole blood samples in EDTA
- Elevated levels of HbF may lead to underestimation of HA1c and, that uremia does not interfere with HbA1c determination by immunoassay. Labile intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunoassay.
- Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

PERFORMANCE CHARACTERISTICS

The following HbA1c value data were obtained by comparing Archem Direct Turbidimetric HbA1c assay to a legally marketed HPLC method.

	Whole blood application
n	44
Slope	1.0212
Intercept	0.0135
Correlation coefficient	0.9874
Range of values	5% - 13% HbA1c

Low Linearity (LOQ) (Based on ARCHITECT c Systems, Also LOQ values are based on CV% values lower than 20%): 4.5% HbA1c. Considerable variation may be seen in linearity depending on the analyzer model and application method.

Sensitivity (LOD) (Based on ARCHITECT c Systems): 3.5%.

High Linearity: Archem HbA1c assay has a linear range from 4.5% - 16 %.

Precision Studies (Based on CLSI EP5 Doc.):

Repeatability (Within Run)(intra-assay): The within run precision was established by ARCHITECT c Systems assaying two blood.

Level	Mean	Std.Dev.	CV%
Low	5.7 %	0.074	1.26
High	11.8 %	0.12	1.73

Reproducibility (Run to Run)(inter-assay): The between day precision was established by ARCHITECT c Systems assaying two blood.

Level	Mean	Std. Dev.	CV%
Low	5.46 %	0.156	2.81
High	10.1 %	0.268	2.72

Correlation: Corr with a reference method is: r=0.99 (Between 5% to 15%)

Trueness: No systematic differences seen in results obtained with this reagent when compared with reference reagents. It's available to get details of comparison experiments in case of requirement.

Interference: The assay is not affected by the following interfering substances at the indicated concentrations:
Ascorbic acid 40 mg/dL,
Total bilirubin 48 mg/dL,
Acetylated Hb to 4,8 mmol/L,
Triglyceride 2000mg/dL,
Carbamylated Hb to 7,3 mmol/L.

Stable glycated hemoglobin serves as a substrate for Turbidimetric reaction used in the Archem Direct Turbidimetric HbA1c assay.

NOTE

Human specimens and all materials that are in contact with samples should be handled and disposed of according to local and national laws and as if such samples are capable of transmitting infection.
1) Reagent R1 and R2 are light-sensitive. Store in a dark place.
2) Specimens containing human sourced materials should be as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Bio-medical Laboratories (HHS Publication Number [CDC] 93-8395).
3) As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4) Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
5) Do not use the reagents after the expiration date labeled on the outer box.
6) Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow. Perform the test according to the general GLP guidelines.

ABBREVIATIONS

CLSI : Clinical and Laboratory Standards Institute
CV% : Coefficient of Variation Percentage
EP : Evaluation Protocols
GLP : Good Laboratory Practice
IU : International Unit
mA : miliabsorbance
mL : mililiter
NCCLS : National Committee for Clinical Laboratory Standards
QC : Quality Control

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SYMBOLS

IVD	Only for invitro diagnostic use
LOT	Lot of manufacturing
R1	Reagent 1
R2	Reagent 2
CONC	Concentration
INGRED	Reagent Ingredients
REF	Reference Number (Catalog No)
SN	Serial Number
	Expiration date
	Storage temperature interval
	Read the directions
	Biological risk

CE

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