HbA_1C DIRECT
HbA_1C DIRECT TURBIDIMETRY

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.

Ref No	Pack	Ref No	Pack	Ref No	Pack	Ref No	Pack
HG200	108 ml	RHB1	2286 Tests	SHB1	490 Tests	LHB3	522 Tests
HG201	54 ml	RHB2	754 Tests	SHB2	343 Tests	LHB4	348 Tests
THB1	2243 Tests	NHB1	552 Tests	MHB1	2727 Tests	BY9000	2573 Tests
THB2	1063 Tests	NHB2	414 Tests	MHB2	1091 Tests	BY9001	1715 Tests
THB3	531 Tests	KHB1	696 Tests	LHB1	2087 Tests		
DMHB10	720 Tests	KHB2	2783 Tests	LHB2	1043 Tests		

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 intended to aid in the monitoring of long-term blood glucose control and compliance in individuals with diabetes mellitus. Archem HbA1c assay is not intended for use in diagnosing diabetes mellitus.

SUMMARY AND EXPLANATION OF TEST

The Archem HbA1c assay measures 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. Several studies, including the Diabetes Control and Complications Trial (DCCT), have shown that long-term control of diabetes can prevent these complications. Therefore, measurement of percent HbA1c can be invaluable in the monitoring of long-term glycemic control of diabetic patients. As of 2008, the ADA does not recommend HbA1c for diagnosis of diabetes due to lack of evidence on prognostic significance and diagnostic thresholds. 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 Hb A1c. The stable HbA1c does not fluctuate in response to rapid changes in physiological factors, and therefore, provides a measure of the individual's mean glucose blood level for the previous several months.

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.1-3 Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months

ASSAY PRINCIPLE

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.

REAGENT COMPOSITION

Lysis Buffer	Reagent R1	Reagent R2(mix):
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.

REAGENT PREPARATION

For analyzers capable of handling 3-reagents, R1a, R1b, R2 are ready to use. **For analyzers capable of handling only 2-reagents, Archem HbA1c reagents R1a and R1b should be mixed in a 100:5 ratio** (Eg: 20ML + 1ML)To prepare sufficient R1ab mixture, pour the entire contents of R1b bottle into R1a bottle. Mix gently by inversion.

REAGENT STABILITY AND STORAGE

Reagents are stable until their expiration date when stored at 2-8°C.

Reconstituted R1ab thus prepared is stable for 1 month when stored at 2-8°C. **R1 and R2 reagents are light sensitive.**

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.

TEST PROCEDURE

Whole Blood Bench Top Lysis Procedure

1) Dispense 250 µL of Lysis reagent in a sample cup or an Eppendorf microfuge tube.

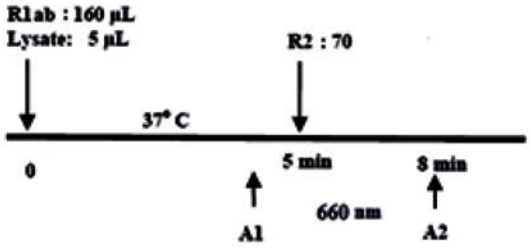
2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to resuspend settled erythrocytes. **Ac-curacy of the assay will be affected if whole blood is not mixed prior to testing.** Add 5 µL of fully resuspended whole blood sample to the lysis buffer in the sample 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 7 days at 2-8 °C.

3) The calibrators and controls should be treated exactly as patient samples and used per instructions on labeling.

4) Direct Turbidimetric HbA1c assay reagents are comprised of redox balanced components.

ASSAY SCHEME FOR ANALISERS

For analyzers capable of handling only 2-reagents, please premix R1a and R1b as described in reagent preparation section and 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 weekly (168 hours) 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. 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 14 days when capped tightly and stored at 2-8°C.

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.

To ensure adequate quality control, level 1 and level 2 controls with known values should be run as unknown samples.

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 NGSP8 format. No calculation step is needed.

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The International Federation of Clinical Chemistry (IFCC) values can be calculated by use of published10.11.
Conversion formula:

NGSP = [0.915 x (IFCC)] + 2.15.

REFERENCE INTERVALS (NORMAL VALUES) (Based on rules CLSI C28-P Document)*

Expected Values: %4.5 - 6.5 (NGSP/DCCT)
Expected Values: %4.5 - %7(Diabetics)(NGSP/DCCT)
Expected Values: 27-46 mmol/mol (IFCC)

Recommended Values: less than 6.5% for a non-diabetic, less than 7% for glycemic control of a person with diabetes. 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.

*Each laboratory should establish its own expected values. In using

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

(Determined on Hitachi 917 chemistry analyzer)

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

PERFORMANCE CHARACTERISTICS

High Linearity / Low Linearity: Archem HbA1c assay has a linear range from 2% - 16.0%.

Precision Studies (Based on CLSI EP5 Doc.):

Repeatability (Within Run)(intra-assay): The within run precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std.Dev.	CV%
Low	5.46	0.074	1.45
High	10.1	0.169	1.73

Reproducibility (Run to Run)(inter-assay): The between day precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

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

Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

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 R1b 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 “Good Laboratory Practice” (GLP) guidelines.

Materials Required but not Provided

1) HbA1c calibrator set Intended for use only with Direct Turbidimetric HbA1c Assay reagents

2) HbA1c calibrator set Intended for use only with Direct Turbidimetric HbA1c Assay.

3) Bi-level HbA1c controls Whole blood hemo-lysates and stabilizers. -

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
SN	Serial Number
REF	Reference Number (Catalog Number)
	Expiration Date
	Storage temperature interval
	Read the directions
	Biological risk

CE

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