HBA1C 2A

IMMUNOTURBIDIMETRY

Intended use:

This reagent is intended for *in vitro* quantitative determination of HbA1c in human blood. Latex enhanced Immunoturbidimetric Ready to use liquid stable reagents. Multipoint calibration: Direct result (% HbA1c) from analyzer. No total Hb determination required.

Clinical Significance:

HbA1c is a glycated form of hemoglobin formed by the attachment of glucose residues in the blood to the hemoglobin molecules. In the diabetic population where blood glucose levels are abnormally elevated the level of HbA1c also increases. The level of HbA1c is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the mean blood glucose concentration in the preceding 6-8 weeks. It is therefore a long-term indicator of diabetic control. For routine use HbA1c levels should be monitored every 3-4 months. However in gestational diabetes and after a change in therapy it may be useful to measure HbA1c more frequently at 2-4 week intervals.

Principle:

The principle of the test is latex agglutination method that measures the ratio of hemoglobin A1C that occupy in total hemoglobin in the whole blood. The sample (hemolysis sample) is added to the sensitized latex particles, and the surface of the latex adsorbs total hemoglobin in the sample. Anti-human HbA1c Mouse monoclonal antibody complex agglutination by anti-mouse IgG antibody. At this time, the amount of agglutination caused depends on the amount of HbA1c that adsorbs the surface of the latex, this this agglutination is measured as a turbidity. The concentration of HbA1c (%) in the sample is determined by referring to the calibration curve obtained by the same test of diluted standard solutions.

Reagent Composition:

HbA1c Direct R1	**1 x 15 mL**	**2 x 15 mL**
Latex		
HbA1c Direct R2	**1 x 5 mL**	**2x 5 mL**
Anti-human HbA1c mouse monoclonal antibody		
Anti-mouse IgG goat antibody		
HbA1c R3	**1 x 32 mL**	**1 x 63 mL**
Hemolysis Reagent		
HbA1c DIRECT CALIBRATOR	**4 x 0.5 mL**	
HbA1c 4 Level Calibrator (Lyophilized)		

Reagent Stability and Storage:

The sealed reagents are stable up to the expiry date stated on the label, when stored at 2- 8, protected from light. Once opened the reagent is stable up to four weeks, if contamination is avoided. Recalibration is recommended after 30 days. **DO NOT FREEZE**

Calibrator: Reconstitute the calibrator with 0.5mL distilled water. It is stable for 30 days at 2-8 . **DO NOT FREEZE.**

Precaution:

To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent and standard bottles immediately after use. Avoid direct exposure of working reagent to light.

Assay General Parameters:

Reaction Mode	End point
Reaction Direction	Increasing
Wavelength	660 nm
Linearity	16%
Blank Method	Reagent blank
Sample volume	5 µL
Reagent 1 Volume	200 µL
Reagent 2 Volume	66 µL

Test Procedure (for Auto Analyzers):

	Blank	Calibrator	Sample/Control
HbA1c 2A R1	1000 µL	1000 µL	1000 µL
Calibrator	-	25 µL	-
Hemolysate(sample/control)		-	25 µL
Mix & incubate for 5 min at 37C.			
HbA1c 2A R2	333 µL	333 µL	333 µL
Mix and incubate for 5 min at 37oC and read absorbance (A) at 660 nm.			

Calculation:

Calibration curve

Calculate the Abs of calibrators = Abs calibrator – Abs Blank. Plot the D Abs of each calibrator versus assigned concentration (HbA1c %) on a linear graph paper. HbA1c results according to NGSP for the samples and controls are determined using the prepared calibration curve.

Calculate

Abs of sample = abs Sample - abs Blank.

HbA1c % in the sample is calculated by interpolation of Abs of sample on the calibration curve. For calculation of results according to IFCC, use IFCC calibrator values (see calibrator insert), or use following equation.

NGSP = (0.915 x IFCC) + 2.15

Working Procedure:

Whole Blood Benchtop Lysis Procedure

1) Dispense 1000µL of Lysis reagent in a sample cup a 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 thoroughly mixed prior to testing. Add 20 µL of fully resuspended whole blood sample. Mix gently with a suitable pipette without creating foam and incubate at room temperature (25°C) for 5-10 min 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 HbA1c assay steps and is stable up to 4 hours at room temperature.

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

Whole Blood On board Lysis Procedure (For supported Auto analyzers)

1) Load HbA1c assay reagents (Lysis Buffer, R1 and R2) on assigned positions on supported auto analyzers reagent trays.

2) Prior to testing, whole blood samples should be mixed by gentle inversion at least 5 times to re suspend settled erythrocytes. Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing. Carefully add at least 100 µL of fully re suspended whole blood sample into auto analyzers sample tube without creating foam or bubble and load on auto analyzers sample tray. Start to run within 5 minutes from adding the first whole blood sample into sample tube. Do not test more than 10 samples in one run, due to settlement of the red blood cells.

Calibration:

The BIOANALYTIC 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. BIOANALYTIC HbA1c calibrator sets are intended for use with Hemoglobin A1c reagents all calibrator vials are stable until their expiration date when stored at 2-8°C. BIOANALYTIC HbA1c calibrator set is in lyophilized form. BIOANALYTIC HbA1c calibrator set for the Supported auto analyzers On-Board Lysis Application includes four levels of calibrator material. Level 0 is in liquid form and ready to use, 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 28 days when capped tightly and stored at 2-8°C. The liquid form calibrator zero is stable for 14 days after opening the vial when capped tightly and stored at 2-8°C.

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Quality Control:

BIOANALYTIC HbA1c control set can be purchased separately. Users should follow the appropriate federal, state and 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.

Reference Range:

Reference normal value (NGSP): 4.0% -6.2%

Non-diabetic individuals have HbA1c values in the range of 4.0 – 6.2% and controlled diabetic individuals have HbA1c values in the 6.5% – 7.5% range. Individuals with uncontrolled diabetes can have HbA1c as high as 20%. The American Diabetes Association (ADA) recommends that the primary treatment goal in diabetes should be glucose control equal to that achieved during the DCCT. Based on DCCT, ADA states HbA1c targets of <7% However, each laboratory must establish its own normal range in their country of business taking into account sex, age and ethnicity.

Linearity:

BIOANALYTIC HbA1c assay has a linear range from 4.0% - 16.0%.

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%).
- The assay is formulated for use with human whole blood samples in EDTA. Total hemoglobin in the sample should be in the range: 9-21 g/dL
- High Hb (>10%) may result in inaccurate HbA1c values.

Performance Characteristics:

The following HbA1c value data were obtained by comparing BIOANALYTIC 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	4% - 16% HbA1c

Precision:

Precision studies were conducted with the BIOANALYTIC HbA1c assay reagents. Within-run and total precision studies were done by testing 2 levels of samples per NCCLS EP-5 procedure. Precision data is summarized in the table below:

	Level 1 (%HbA1c)	Level 2 (%HbA1c)
Mean value	5.7%	13.3%
Within run SD (Swr)	0.16	0.37
Within run CV%	1.0%	0.7%
Inter assay precision	0.10	0.18
Inter assay precision	1.8%	1.8%

Interference:

The assay is not affected by the following interfering substances at the indicated concentrations: ascorbic acid 12 mg/dL, total bilirubin 15mg/dL, bilirubin (conjugated) 13mg/dL, glucose 4000mg/dL, triglyceride 4000mg/dl, uric acid 30 mg/dL, urea 80mg/dL.

References:

a) Goldstein, D.E. *et al,* Diabetes Care. 27(7):1761-73 (2004)
b) United Kingdom Prospective study, Lancet 352: 837-53 (1998)
c) Little, R. *et al.*, Clin Chemistry, 47: 1985-1992 (2001)
d) American Diabetes Association. Clinical practice recommendation: standards of medical care for patients with diabetes mellitus. Diab Care22 (supp): S32-41 (1999)
e) American Diabetes Association Clin. Practice recommendation, 1992, Diab Care 16S2 (93): 10-13
f) NGSP, http://www.missouri.edu/~diabetes/ngsp.html
g) Goldstein *et al*, Clin Chem 32: B64-B70 (1986)
h) Hoelzel W *et al*. IFCC reference system for measurement of hemoglobin A1C in human blood and the national standardization schemes in the United States, Japan and Sweden: a method-comparison study. Clin Chem 2004;50:166-74
Sacks, D (ed). Global Harmonization of Hemoglobin A1C. Clin Chem 51(4): 681-683 (2005)

Order information (Cat No.) :

OL505	B22175	B27176	B30177	B33176	B36176
CC508	B24175	B27177	B31175	B33177	B36177
AB509	B24176	B28175	B31176	B34175	B37175
KL505	B25175	B28176	B32175	B34177	B37176
B21175	B25176	B28177	B32176	B35175	B37177
B21176	B25177	B30175	B32177	B35176	B80175
B21177	B27175	B30176	B33175	B36175	

Manufacturer

Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti
Adress : İkitelli O.S.B Mutsan San.Sit. M4 Blok No:17-19 Başakşehir/İSTANBUL
Tel:+90(212) 549 33 88- Fax:+90 (212) 549 55 50
Web :www.diaclinica.com

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month
	warning, read enclosed documents
	Read the directions

ISO 9001:2015
ISO 13485:2016