HbA1c Reagent Kit
CliniQuant – FSR

Meril Diagnostics

IVD For *in vitro* diagnostic use
Read this pack insert thoroughly before use

REF	Pack size R1 HbA1c Reagent	Pack size R2 HbA1c Reagent	Pack size R3 HbA1c Reagent	Pack size R4 Calibrators
HBATIA-01	2 x 15ml	2 x 5ml	2 x 50ml	4 x 1.0 ml
HBATIA-02	1 x 15ml	1 x 5ml	2 x 25ml	4 x 1.0 ml

INTENDED USE

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

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.

PRINCIPLE OF THE METHOD

The whole blood is lysed using haemolysing reagent. The lysed whole blood containing HbA1c along with other haemoglobins compete to adsorb to the unsensitised latex particles in the R1. A mouse antihuman HbA1c monoclonal antibody is added into the reaction that specifically binds to the human HbA1c molecules to form latex HbA1c- mouse antihuman HbA1c antibody complex. Another antibody, goat anti mouse polyclonal antibody that react with the formed complex to give agglutination. The amount of agglutination is proportional to the amount of HbA1c adsorbed on to the surface of latex particles. It is measured at 670 nm which is used to calculate the HbA1c % from a calibration curve.

KIT COMPONENTS

Composition :
R1 - HbA1c Reagent : Latex, Glycine Buffer
R2 - HbA1c Reagent : HbA1c Direct Anti-human HbA1c mouse mono clonal antibody, Anti-mouse IgG goat antibody
R3 - HbA1c Reagent : Surfactant
R4 - Calibrators : 4 level calibrators. Human RBC (Lyophilized). Concentration is lot specific, see vial labels.

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettes & Tips, Test Tubes & Racks, Timer, Incubator, Control Level 1 & 2, Analyzer.

REAGENT STORAGE & SHELF-LIFE

The reagents are ready to use.
The sealed reagents are stable up to the expiry date stated on the label, when stored at 2-8°C and protected from light.
DO NOT FREEZE.
Once opened, the reagent is stable for 4weeks at 2-8°C, if contamination is avoided.

Calibrator: Reconstitute the calibrator with 1.0 ml DI water, allow to stand for 15 minutes at room temperature. The reconstituted calibrators are stable up to 15 days at 2-8°C.
Use the reconstituted calibrator directly, do not follow further haemolysing step. DO NOT FREEZE.

SAMPLE/ CONTROLS

EDTA-Whole Blood. To determine HbA1c, a haemolysate must be prepared for each sample.
Haemolysis:
1. Dispense 0.5 ml of haemolysing reagent (R3) in to a tube
2. Add 10 µl of well mixed whole blood and control.
3. Mix the reactants
4. Allow to stand for 15 minutes at room temperature for complete lysis.

Follow the same procedure with sample and controls.

REAGENT DETERIORATION

Turbidity or precipitation in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the HbA1C control may also be an indication of reagent instability and associated results are invalid. Sample should be retested, using a fresh vial of reagents.

WARNINGS AND PRECAUTIONS

1. To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent and calibrator bottles immediately after use. Do NOT FREEZE the reagent as freezing can decrease the reagent activity.
2. Avoid direct exposure of working reagent to sun light.
3. Specimens should be considered infectious and handled appropriately.
4. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

Programme Parameter for MERILYZER CliniQuant/ CliniQuant Micro

	MERILYZER CliniQuant	CliniQuant Micro
Reading Mode	Multi Standard	Multi Standard
Calibrator Conc.	(%) See vial label	(%) See vial label
Filter - 1 (nm)	670	620
Filter - 2 (nm)	-	-
Temperature	37 °C	37 °C

Meril Diagnostics Pvt. Ltd., Second Floor, D1-D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. T+91-260-3052100, F +91-260-3052125, W www.merillife.com

Volume (µl)	400	400
Delay Time (Sec)	5	5
Unit	%	%
Reaction Direction	Increase	Increase
Reference Low:	4.6%	4.6%
Reference High:	6.2%	6.2%
Linearity	Upto 16%	Upto 16%

TEST PROCEDURE

Dispense	Blank	Calibrator	Sample
Reagent 1	-	300 µl	300 µl
Calibrator	-	10 µl	-
Sample	-	-	10 µl
Mix, incubate for 5 min at 37°C.			
Reagent 2	-	100 µl	100 µl
Mix, incubate for 5 min at 37°C. **Read absorbance of calibrator & sample against water blank at 670 nm.**			

CALCULATION & PREPARATION OF CALIBRATION CURVE

Prepare a calibration curve using 4 level calibrator provided in the kit.
Delta Abs. of calibrators=Abs. of calibrator–Abs. of blank.
Plot the Delta Abs. of each calibrator versus assigned concentration (HbA1c %) on a linear graph paper.

Calculate Delta Abs. of sample i.e. Abs. of sample – Abs. of blank. HbA1c % in the sample is calculated by interpolation of Abs. of sample on the calibration curve.

Note: Calibration on Merilyzer CliniQuant has programming facilities to plot the calibration curve. HbA1c results according to NGSP for the samples and controls are determined using the calibration curve.

For calculation of results according to IFCC, use equation.
NGSP = (0.915 x IFCC) + 2.15.

LIMITATIONS

Low total hemoglobin samples (< 8 g/dl) may show low HbA1c results.

QUALITY CONTROL

It is recommended to use commercial third party HbA1c Control level 1&2 to verify the performance of the assay. Each laboratory has to establish its own internal quality control scheme and procedure for corrective action, if controls do not recover within the acceptable tolerance.

REFERENCE RANGE

It is recommended that each laboratory establish its own reference values. The following value may be used as guide line.
Reference normal value: 4.6% - 6.2% HbA1c

PERFORMANCE CHARACTERISTICS

1. Linearity

This reagent is linear upto 16% HbA1c (NGSP). Sample above the measuring range should not be diluted and retested. These samples should be tested with alternative methods.

2. Sensitivity/ Limit of detection (LOD)

Lower detection limit (or analytical sensitivity) of the assay is 3 %.

3. Interferences

No interference for: Ascorbic acid up to: 50 mg/dl
Bilirubin up to: 40 mg/dl; Intra lipid up to: 3000 mg/dl

It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead poisoning, alcoholism and ingestion of large doses of aspirin. Elevated HbF levels may lead to under estimation of HbA1c.

4. Precision

Intra-assay precision

	Mean	SD	CV
n = 20	%	%	%
sample 1	6.16	0.07	1.10
sample 2	9.53	0.1	1.03

Inter-assay precision

	Mean	SD	CV
n = 20	%	%	%
sample 1	6.17	0.07	1.06
sample 2	9.56	0.11	1.10

5. Methods Comparison

A comparison study has been performed between CliniQuant FSR – HbA1c reagent and another internationally available reagent yielded a correlation coefficient of r2 = 0.9884 and a regression equation of y = 1.0014x.

WASTE DISPOSAL

This product is made to be used in professional laboratories.
Please consult local regulations for correct waste disposal.

REFERENCES

1. Engbeak, F., et al. Clin chem.35 pp. 93-97 (1989).
2. American Diabetes Association: Clinical practice recommendations (position statement). Diabetes care 24 (suppl.1) S33-S55, (2001).
3. Tietz, N.W. Textbook of Clinical Chemistry, W.B. Saunders Company, p.794 - 7795 (1999).

IFU/HBATIA01/03
05-08-2019

Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged