8. All HbA1c methods are inadequate for the assessment of long-term glycemic control in patients homozygous for HbS, HbC or HbSC disease.
9. Immunoassays and boronate affinity methods have been shown to be unaffected by concentration of carbamyl-Hb encountered in ureamic patients[6].
10. A variety of patient-related factors and laboratory related processes can lead to inaccurate determinations of HbA1c in the setting of variant Hbs. Samples should be evaluated for the presence of a Hb variant with any HbA1c readings > 15%[6].
11. Patients with elevated levels of HbF may demonstrate lower HbA1c values.
12. High HbA1c levels should not be the only basis for treatment. Values should be correlated with clinical Picture.
13. The measuring range of the assay is as indicated in the pack insert. Values outside the measuring range are extrapolated values and should not be considered as accurate.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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9. Data on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

	Temperature limitation		Manufacturer		This way up	SC	Smart card (RFID)
	Use by		Consult Instructions for use	HS	Hemolysing Solution	CT	Cuvettes
	Date of Manufacture	REF	Catalogue Number	Σ	Contains sufficient for <n> tests	R1	Latex Reagent
LOT	Batch Number/ Lot Number	IVD	*In vitro* Diagnostic Medical Device	EC REP	Authorised Representative in the European Community	R2	Antibody Reagent

TULIP DIAGNOSTICS (P) LTD.

REGD. OFFICE: GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA. Website: www.tulipgroup.com

MANUFACTURING UNIT: PLOT NOS. 92/96, PHASE II C, VERNA IND. EST., VERNA, GOA-403 722, INDIA.

EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

1221/VER-01

CE

turbosmart™

HbA1c

IMMUNNOTURBIDIMETRIC ASSAY FOR DETERMINATION OF HbA1c IN HUMAN BLOOD ON TURBOSMART™

SUMMARY

Glycated hemoglobin (GHb) also commonly known as glycosylated hemoglobin, glycohemoglobin, HbA1, HbA1c or A1c is a term used to describe a series of stable minor hemoglobin components formed slowly and non-enzymatically from hemoglobin and glucose. The glycation of hemoglobin can occur at various sites present on the polypeptide chains of the hemoglobin molecule with different carbohydrate (sugar) molecules. The glycohemoglobin is subdivided into subfractions depending on each of the glycation sites and reaction partners involved in glycation. More recently HbA1c is defined as Hb that is irreversibly glycated at one or both N-terminal valines of the β-chain. The remaining GHbs have glucose, glucose-6-phosphate, fructose-1, 6-diphosphate, or pyruvic acid bound to one of the 44 additional sites occurring at e-amino group of lysine residues or at the NH_2 terminal of the α-chain. Formation of HbA1c is irreversible and the blood levels depend on both the life span of the red blood cell (average 120 days) and the blood glucose concentration. The rate of formation of HbA1c is directly proportional to the ambient glucose concentration. The amount of HbA1c therefore represents the integrated values of glucose over the preceding six to eight weeks and provides an additional means of assessing glycemic control. The results of HbA1c are not influenced by recent meals, physical activity or emotional stress.

Maintaining glycemic levels as close to diabetic range as possible has been demonstrated to have a powerful beneficial impact on diabetes-specific complications, including retinopathy, nephropathy and neuropathy in the setting of type 1 diabetes; in type 2 diabetes, more intensive treatment strategies have likewise been demonstrated to reduce complications. Intensive glycemic management resulting in lower HbA1c levels has also been shown to have a beneficial effect on cardiovascular disease complications in type 1 diabetes.

The measurement of HbA1c in human blood is therefore considered the most important marker for long-term assessment of glycemic state in patients with diabetes, and goals for therapy are set at specific HbA1c target values.

The two seminal studies, The Diabetes Control and Complications trial (DCCT) and the United Kingdom Prospective Diabetes study (UKPDS) proved the usefulness of HbA1c measurement in predicting the risk of developing microvascular complications and, as a consequence, have led to the widespread recommendations of its increased use.

turbosmart™ HbA1c is a turbidimetric immunoassay for the direct determination of HbA1c in human blood without the need to estimate total hemoglobin.

PRESENTATION

REF	108780020	108780060
Σ	20 Tests	60 Tests
R1	20 Tests	3 x 20 Tests
R2	20 Tests	3 x 20 Tests
HS	10 ml	10 ml
SC	1 No.	1 No.
CT	20 Nos.	60 Nos.

REAGENT

turbosmart™ HbA1c assay contains:

1. R1 **turbosmart™ HbA1c Latex Reagent (R1):** ready to use uniform suspension of latex particles.
2. R2 **turbosmart™ HbA1c Antibody Reagent (R2):** ready to use solution of mouse anti human HbA1c and goat anti mouse IgG antibody.
3. HS **turbosmart™ HbA1c Hemolysing Solution:** ready to use solution.
4. SC **turbosmart™ HbA1c RFID:** Card with HbA1c Master Calibration curve calibrated with a standards traceable to a NGSP (National Glycohaemoglobin Standardisation program) certified method that has documented traceability to the Diabetes Control and Complications Trial (DCCT) reference method.
   1NGSP A1c = 0.915 (IFCC A1c) + 2.15
   IFCC A1c = (NGSP A1c-2.15)/0.915

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent and activation buffer is as per the expiry date mentioned on the respective vial labels.
3. Once opened the reagents are stable for 75 days when stored at 2-8°C provide the reagents are not contaminated.
4. Store the **turbosmart™** RFID card at a clean dry place. The **turbosmart™** RFID card data once transferred into **turbosmart™** analyzer is valid upto the use of labelled number of tests within 75 days.

PRINCIPLE

turbosmart™ HbA1c is a turbidimetric immunoassay for direct determination of HbA1c and is based on the principle of agglutination reaction. The test specimen after treatment with Hemolysing solution is allowed to react with latex reagent (R1). Total Hb and HbA1c bind with same affinity to latex particles. The amount of binding is proportional to the relative concentration of both substances in blood. The reaction mixture is then allowed to react with mouse anti human HbA1c monoclonal antibody & Goat anti mouse human IgG (R2) resulting in agglutination reaction that is measured at ~ 650 nm. The increase in turbidity corresponds to the concentration of HbA1c in the test specimen.

NOTE

1. In vitro diagnostic reagent for professional use only. Not for medicinal use.
2. All reagents derived from human source have been tested for HbsAg and HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.09% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. Gently mix the **turbosmart™ HbA1c** latex reagent well before use to achieve optimum test performance.
5. **As the reagents and RFID card within lots have been matched, reagents or RFID cards from different lots must not be interchanged.**
6. The reagent performance must be validated periodically with known controls such as **Turbodyne™ HbA1c** control (Ref 108600002).
7. Do not use damaged or leaking reagents.
8. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures.
9. **Always use fresh clean disposable micropipette tips to aspirate the reagents to prevent contamination.**

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection by approved techniques. No special additives or preservatives other than anticoagulants are required. Collect venous blood in EDTA using aseptic techniques.

Specimen preparation:

1. Mix the specimen (sample or reconstituted calibrator or reconstituted control) thoroughly to obtain uniform distribution of erythrocytes. Avoid bubble formation.
2. Take 500µl **turbosmart™ HbA1c** Hemolysing solution in a test tube.
3. Add 10µl of homogenised specimen (sample/reconstituted calibrator/reconstituted control). Mix well and allow to stand for **15 minutes** or until complete lysis is apparent. This hemolysed specimen is referred as **Lysate**.

Specimen stability:

Whole blood: 1 week at 2-8°C, Lysate: 10 hours at 15-25°C Lysate: 10 days at 2-8°C

ADDITIONAL MATERIAL REQUIRED

turbosmart™ analyser, stopwatch, well calibrated micropipettes, disposable tips, incubator.

TEST PROCEDURE

1. Bring reagent and sample to room temperature before use.
2. Select the HbA1c test from the Measure Menu of Instrument.
3. Load the **turbosmart™ HbA1c** test data from the RFID card (Provided with the kit) to the analyser as described in the Instrument User Manual. The Instrument is ready to perform the HbA1c test.
4. The instrument will indicate to place cuvette with R1 + sample in the reading chamber.
5. Take a disposable cuvette (provided in the kit) and add 180µl R1 to the cuvette using fresh clean disposable micropipette tips.
6. Then add 5µl sample and incubate the cuvette for 5 minutes.
7. Place the cuvette with R1 + sample in the **turbosmart™** reading chamber.
8. Long Press "Test"key. The instrument will mix the sample and then indicate to add R2.
9. Long press **turbosmart™** electronic Pipette to dispense 60µl R2 reagent to the cuvette with R1+sample.
10. The reaction will start and the counter will start in the display. Results will be displayed on completion of reaction.

The obtained % HbA1c values can also be converted into eAG (mg/dl), eAG (mmol/l), IFCC HbA1cas descried below:

HbA1c	Estimated average glucose (eAG)		IFCC HbA1c	Interpretation
%	Mg/dl	Mmol/L	Mmol/mol	
4	65	3.5	20	Non-diabetic range
5	100	5.5	31	
6	135	7.5	42	
7	170	9.5	53	ADA target
8	205	11.5	64	Action suggested
9	240	13.5	75	
10	275	15.5	86	
11	310	17.5	97	
12	345	19.5	108	

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring Range

The **turbosmart™ HbA1c** has been designed to measure HbA1c concentrations in the range of 5 -15%. The exact range is dependant on the calibrator value used for calibration which is lot specific.

Detection limit / Analytical Sensitivity

The limit of detection is 5 % HbA1c. The detection limit represents the lowest measurable HbA1c concentrations that can be distinguished from zero.

Precision

Intra-assay precision	n	Mean %	SD	CV (%)
Sample 1	10	6.0	0.1	2.2
Sample 2	10	8.0	0.2	2.1
Sample 3	10	10.1	0.2	2.3

Inter-assay precision	n	Mean %	SD	CV (%)
Sample 1	10	6.1	0.2	3.2
Sample 2	10	8.0	0.2	2.9
Sample 3	10	10.0	0.2	2.4

Interference

Bilirubin upto 60 mg/dl, Ascorbic acid upto 60 mg/dl, Triglycerides upto 1200 mg/dl, RF upto 700 IU/ml, Carbamylated Hb upto 7.5 mmol/L and Acetylated Hb upto 5 mmol/L, do not Interfere in this assay.

REFERENCE INTERVAL

1.Non-diabetics < 6%

For Glycemic control in diabetics < 7%. A level > 7% indicates persistent glycemia over previous 6-8 weeks indicating poor diabetes management.

Each laboratory should determine its own reference interval on a non-diabetic population. When using HbA1c for monitoring glycemic control in diabetics, results should be interpreted individually. The HbA1c values must be compared with previous HbA1c values from the same patient using the same assay system.

REMARKS

1. Any situation that shortens erythrocytes survival or decreases mean erythrocyte age falsely lowers GHb (HbA1c) test results regardless of the test method.
2. Iron deficiency anemia is reported to increase test results that can subsequently be reversed by iron treatment. Hemolytic anemia has opposite effect to iron deficiency by reducing HbA1c in affected individuals.
3. It has been reported that results may be inconsistent in patients who have conditions such as opiate addiction, lead poisoning, and alcoholism, ingest large doses of Aspirin.
4. It is reported that Antibodies used in immuoturbidimetric assays recognize the N-terminal glycated amino acid in the context of 4 -10 amino acids of the Hb β-chain.These antibodies do not recognize the reversible Schiff base (aldimine) or other gHb species, including chemically modified derivatives[8].
5. HbE has little effect on the determination of glycosylated hemoglobin with immunoassay methods.
6. It has been documented that HbF has little or no immunoreactivity with most antibodies used in HbA1c assays.
7. Any patient with a significant change in HbA1c coinciding with a change in laboratory HbA1c methods should be evaluated for the presence of variant or derivative Hb.