Turbidimetric immunoassay for quantitative determination of HbA1c in human blood

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 ß-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 α-amino group of lysine residues or at the NH_2terminal 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.

Quantia®-HbA1c PLUS is a turbidimetric immunoassay for the direct determination of HbA1c in human blood without the need to estimate total hemoglobin.

REAGENTS

1. **Quantia®-HbA1c PLUS** Latex Reagent (R1): ready to use uniform suspension of latex particles.
2. **Quantia®-HbA1c PLUS** Antibody Reagent (R2): ready to use solution of anti human HbA1c monoclonal antibody.
3. **Quantia®-HbA1c PLUS** Hemolysing Solution: ready to use solution.

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

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8^{0}C. DO NOT FREEZE.
2. The shelf life of the latex reagent (R1), antibody reagent (R2) and Hemolysing solution is as per the expiry date mentioned on the respective vial labels.

PRINCIPLE

Quantia®-HbA1c PLUS 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 anti human HbA1c antibody (R2) resulting in agglutination reaction that is measured at 630 nm as absorbance. The increase in absorbance 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. How ever 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. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified with **Quantia®-HbA1c** controls (Cat. No.: 1111160002) periodically.
5. Gently mix the **Quantia®-HbA1c PLUS** latex reagent well before use to achieve optimum test performance.
6. Gently mix each of the Quantia reagents to attain homogeneity prior to use for obtaining better performance.
7. Do not use vortex mixtures for mixing reagents. Gently mix the reagents and samples during test procedures.
8. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
9. The **Quantia®-HbA1c PLUS** assay provides optimum results in cuvette mode analyzers. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer.
10. The procedure in this pack insert is designed considering a minimum reading volume of 500µl. In case of instruments where minimum volume required for reading absorbance is 1.0 ml use double the quantity of reagents and samples mentioned in the test procedure.
11. Do not use damaged or leaking reagents.
12. Only **Quantia®-HbA1c** calibrator set (Cat. No.: 1111150302) must be used for calibration of **Quantia®-HbA1c PLUS**. The **Quantia®-HbA1c PLUS** assay is standardised with **Quantia®-HbA1c** calibrator set having values traceable to NGSP (National Glycohaemoglobin Standardisation Program) certified method that has documented traceability to DCCT reference method.

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 **Quantia®-HbA1c PLUS** 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^{0}C
- Lysate : 10 hours at 15-25^{0}C
- Lysate : 10 days at 2-8^{0}C

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 630 nm wavelength filter and cuvette mode, stop watch, well calibrated micropipettes, disposable tips, test tube rack, incubator/waterbath set at 37^{0}C, optically clean disposable cuvettes such as Quantiamate semi micro cuvettes/glass cuvettes, **Quantia®-HbA1c** lyophilised calibrator set (Cat. No.: 1111150302), **Quantia®-HbA1c** lyophilised control (Cat. N1111160002)

TEST PROCEDURE

Bring reagents and samples to room temperature prior to use.
Assay conditions:

Wavelength	630 nm
Reaction temperature	37^{0}C
Optical path length	1 cm

Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.

Test procedure for preparation of calibration curve and specimen.

1. Zero the instrument with distilled water.
2. Pipette out 400µl of **Quantia®-HbA1c PLUS** latex reagent (R1) and add 10µl of lysate. Mix and incubate at 37°C for 5 minutes.
3. Add 100 µl **Quantia®-HbA1c PLUS** Anti HbA1c antibody reagent (R2) and mix well. Immediately start the stop watch.
4. Exactly at 20 seconds take the reading (A1) and at the end of 3 minutes take the reading (A2). Calculate ΔA = (A2-A1).
5. Repeat step 2-4 for all three levels of calibrators. Plot the graph.

Preparation of calibration curve:

Calculate △A (A2-A1) for each calibrator concentration using **Quantia®-HbA1c** calibrator set. Plot a curve of △A versus % HbA1c.

CALCULATIONS

The concentration of HbA1c in the test specimen can be determined directly by interpolating △A of the test specimen from the calibration curve.

The % HbA1c values can also be converted into Mean Plasma Glucose values as described in American Diabetes Association (ADA) recommendations for assays traceable to the DCCT reference method as descried below:

% HbA1c	Approximate mean blood glucose		Interpretation
	Mg/dl	Mmol/L	
4	65	3.5	Non-diabetic range
5	100	5.5	
6	135	7.5	
7	170	9.5	ADA target
8	205	11.5	Action suggested
9	240	13.5	
10	275	15.5	
11	310	17.5	
12	345	19.5	

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range

The **Quantia®-HbA1c PLUS** has been designed to measure HbA1c concentrations in the range of 4 -15% in specimens with haemoglobin concentration between 6 - 23 g/dl.

Detection limit

The limit of detection is 3% 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	6.01	0.14	2.29
Sample 2	10	8.08	0.23	2.81
Sample 3	10	10.06	0.28	2.81

Inter assay precision	n	Mean (%)	SD	CV (%)
Sample 1	10	5.97	0.13	2.24
Sample 2	10	8.07	0.25	3.05
Sample 3	10	10.04	0.16	1.64

Interferences:
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.

METHOD COMPARISON
Quantia®-HbA1c PLUS (y) has been evaluated using 40 samples, in comparison with another commercial immunoturbidimetric assay (x) that is standardized according to approved IFCC reference method. The correlation coefficient (r) was 0.991 and the regression equation (Passing and Bablok) was y=0.99x - 0.071.

REFERENCE INTERVAL
1. Non-diabetics < 6%
2. 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.