HbA1c
QUANTI CARD

Fluorescence immunoassay (antigen-antibody)
for quantitative measurement of HbA1c (Glycated hemoglobin) in human whole blood.

INTRODUCTION
HbA1c (Glycated hemoglobin) is the product of the hemoglobin combining with blood glucose in red blood cells, which can reflect the average blood glucose level 2 -3 months prior to the measurement. HbA1c is the "gold standard" of evaluation for long-term blood glucose controls. HbA1c is considered as a more reliable marker in monitoring glycaemia. The high sensitivity and specificity of HbA1c rapid quantitative test can be achieved by sandwich fluorescence immunoassay technology.

INTENDED USE
HbA1c Quanti Card is a rapid and sensitive immunoassay for the quantitative determination of HbA1c (Glycated hemoglobin) in human whole blood with iQuant Analyzer. The test is used for routine monitoring of long term glycemic status in patient with diabetes mellitus.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)
HbA1c Quanti Card is based on fluorescence immunoassay technology. The test uses anti-HbA1c antibody and anti-hemoglobin antibody immobilized separately on a nitrocellulose strip. Whole blood sample is added to the mixture of HbA1c Conjugate and Assay Buffer, resulting in hemolysis of red blood cells. The mixture containing HbA1c and Hb from the hemolyzed red blood cells and fluorescence labelled HbA1c and Hb from HbA1c Conjugate, is added to the sample well of the cartridge. The mixture migrates through the nitrocellulose strip by capillary action. When the complex meets the line of the corresponding immobilized antibody on nitrocellulose strip, the fluorescence signal is produced from immuno-complex. The signal is interpreted and the result is displayed on i-Quant Analyzer in terms of percentage.

MATERIALS PROVIDED
HbA1c Quanti Card Test kit contains following components to perform the assay:

1. HbA1c Quanti Card (1 Test)	2. HbA1c Conjugate (Dried)	3. Assay Buffer
4. Clamp & Rod	5. Instruction Manual	

MATERIAL REQUIRED, BUT NOT PROVIDED

iQuant Analyzer	Micropipette & Microtips	
Stop Watch	Swab & Lancet	Dual iHeating Block

KIT PRESENTATION
50 Test Pack

STORAGE AND STABILITY
HbA1c Quanti Card should be stored at 2-8ºC in the coolest & driest area available. Expiry date on the kit indicates the date beyond which kit should not be used. The kit should not be frozen & must be protected from exposure to humidity and direct sunlight.

DESCRIPTION OF SYMBOLS USED
The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical cartridges and their packing. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
	Single use only		

WARNING FOR USERS
CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures and guidelines.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. HbA1c Conjugate and Assay Buffer contains Sodium Azide as a preservative. If these materials are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
10. Follow standard biosafety guidelines for handling & disposal of potentially infectious material.

PRECAUTIONS
In order to obtain reproducible results, the following instructions must be followed:

1. Do not use the kit beyond the expiry date.
2. Do not mix reagents from different batches.
3. Do not open the foil pouch of test cards and HbA1c Conjugate vials until it attains room temperature (20-30˚C).
4. Do not open the screw cap of HbA1c conjugate vial until it attains Room Temperature (20-30˚C).
5. Do not re-use the test cartridge.
6. It is recommended to use separate sterile disposable tips for adding Assay Buffer and each sample in order to avoid microbial- contamination of reagents.
7. Follow the given test procedure and storage instructions strictly.
8. Do not paste any sticker or write anything on the QR-Code as this will lead to erroneous result.
9. Do not temper the QR-Code as this will lead to erroneous result.
10. Test cartridge contaminated by blood or other liquid must not be inserted into iQuant Analyzer
11. Sample with extreme Hematocrit i.e. below 25% or over 65% may give erroneous result.
12. Do not use frozen or turbid blood or any artificial material.
13. All pipetting steps should be performed with utmost care and accuracy. Cross-contamination between reagents and samples will cause erroneous results.
14. Do not touch the result reading window of the cartridge this will lead to erroneous result.

Important Note: HbA1c Quanti Card is only operational in connection with iQuant Analyzer.

SAMPLE / SPECIMEN COLLECTION AND STORAGE
1. Collect the whole blood in a clean container (containing EDTA, sodium floride or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8ºC for not more than 1 day, otherwise false / erroneous results may be obtained.
2. Do not use sample with obvious appearance of hemolysis, blood clot or sample with microbial contamination as these samples might interfere the test causing wrong result.
3. Fresh blood from finger prick may also be used as a test sample.

TEST PROCEDURE
RT 20-30ºC

1. Bring the complete kit and sample to be tested to room temperature prior to testing.
2. Remove the test cartridge from the foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test cartridge from the foil pouch.

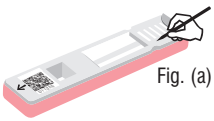


Fig. (a)

3. Label the test cartridge with patient's name or identification number (Fig. (a)). **DO NOT write on QR Code.**
4. Take out required no. of HbA1c conjugate (Dried) vials from the aluminum pouch.
5. Unscrew the HbA1c conjugate vial & add 200µl of Assay Buffer (Use separate HbA1c conjugate vial for each sample). Use fresh/new micropipette tip for each pipetting step. Close the vial cap.

Important Note:

a) Check the presence of Conjugate in the form of color button at the bottom of the vial before addition of the Assay Buffer.

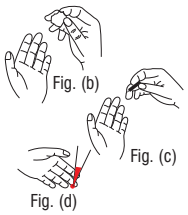
b) Every time HbA1c Conjugate vials are taken out, always reseal the pouch along with desiccant using Clamp & Rod provided and store at 2-8ºC.

c) HbA1c Conjugate (Dried) vials are stable for 30 days at 2-8ºC from the date of opening of seal pouch, when stored with desiccant along with Clamp & Rod.

6. Keep the conjugate vials undisturbed for one minute & then vortex it well for 30 seconds.
7. In case of collected Whole Blood sample, mix the sample properly before use and add 10µl of mixed whole blood sample using the micropipette to above mixture vial. Close the cap of the vial tightly and mix it on iVortexer for 5 - 10 seconds.

OR

In case, finger prick blood is being used, clean the skin surface of the fingertip with the Alcohol swab (fig b) .Let it dry and then prick the lateral part of the fingertip with the sterile Lancet . Make full blood drop and collect 10 μl sample into the micropipette using standard procedure. Immediately add 10 μl of whole blood to the mixture vial of step 4. Close the cap tightly and mix thoroughly by vortexing it on iVortexer for 5 - 10 seconds. (Care should be taken that the blood sample does not clot and then transfer to the Assay Buffer vial immediately). Always store the unused HbA1c conjugate in aluminum foil pouch.



8. Immediately add 75 μl of above mixture using micropipette to the sample well of the cartridge & insert it into the cartridge slot of dual i-heating block. Care should be taken to avoid any spillage on the QR-Code pasted on the cartridge & on the result reading window.

Note : Dispose off the microtip and remaining sample mixture considering them biohazardous.

9. Allow the reaction to occur for 15 minutes. In the meantime enter the patient's details in the iQuant analyzer testing window and select the HbA1c test from the pop down menu in the testing window of the iQuant analyzer.

10. **Insert the test cartridge into the iQuant Analyzer with arrow (←) marked side on the top of cartridge facing towards the analyzer and press RUN button. Note down the value displayed on the screen of iQuant Analyzer.**

11. Discard the HbA1c Quanti Card immediately after reading results at 15 minutes considering it to be potentially infectious.

Important Note: Do not read results after 15 minutes.

MEASURING RANGE

HbA1c (%) : 4 to 16 %

Note:

1 The HbA1c percentage can be converted to m mol/mol (IFCC Unit) by using the following formula: m mol/mol = 10.93 x % - 23.5

2. The average blood sugar level can be estimated based on the measured percentage of glycosylated hemoglobin (HbA1c) using following FORMULA:
Average Plasma Blood Glucose (mg/dl) = (HbA1c * 35.6) - 77.3

DETECTION LIMIT : 4%

Distribution of normal values ranges from 4.5% to 6.5%.

PERFORMANCE CHARACTERISTICS OF HbA1c Quanti Card

1. Precision

Intra-Assay: Within-run and between-run precision have been determined by testing 10 replicates of 5 different samples with HbA1c value (5%, 6.5%, 7.5%, 10.4% & 12.2% respectively) on the same lot on same day. The C.V (%) is $\leq$6%.

Inter-Assay: The inter-assays were performed with 5 replicates of 5 different samples with HbA1c value (5%, 6.5%, 7.5%, 10.4% & 12.2% respectively) of three different lots on 10 sequential days. The C.V (%) is $\leq$6%.

2. Accuracy

The HbA1c concentration of 130 clinical specimen were quantified independently with HbA1c Quanti Card and commercially available NGSP certified kit. The following results were obtained:

Slope : 0.999
Y-Intercept : 0.0176
R^2 : 0.9963

3. Linearity

It is checked by testing 24 HbA1c samples in duplicate covering measuring range 4% to 16% and corelation coefficient (R) is $\geq$0.99.

4. Specificity

No interference with the following hemoglobin Hb variants (HbAC, HbAE, HbAF, HbAV, HbAS, carbamylated and pre-glycated hemoglobin) was observed.

There was no significant interference with the HbA1c measurement when other biomolecules such as Gentisicacid (200 mg/ml), Bilirubin (20 mg/ml), Triglyceride (3000 mg/dl), Ascorbic acid (5 mg/dl) and glucose (300 mg/ml) were added to the test specimen with much higher level than in normal blood.

LIMITATIONS AND INTERFERENCES

1. The test procedure, precautions and interpretation of results for this test must be strictly followed.
2. As with all diagnostic tests, the test result must always be correlated with clinical finding and laboratory data available.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. Technical / procedural errors as well as the presence of additional substances in blood samples may interfere with product performance and may cause erroneous results.
5. The test has been developed for testing human whole blood samples only.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

TROUBLE SHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Unexpected low Test Result	a) Hemolysed sample used. b) whole blood sample not used. c) Insufficient volume of blood sample used. d) Fresh blood sample not used.	Repeat the test using fresh whole blood sample.
	e) Reading has been taken at less than 15 minutes.	Read the result at 15 minutes only.
	f) Reagents used were too cold & were not brought to Room Temperature (R.T.)	Bring the whole test kit to RT before testing.
	g) HbA1c Dried Conjugate vials are continuously exposed to light for more than 8 hours in open condition.	Always keep conjugate vial pouch sealed with clamp & rod and store at 2-8˚C when not in use.
	h) High volume of Assay Buffer used.	Always use calibrated pipette for pipetting Assay Buffer (200 μl)
	i) Expired Test kit used	Repeat the test using a new test kit that has not passed the expiration date.
	j) Improper i.e. less volume of reaction mixture applied to sample well of cartridge	Use appropriate volume (75 μl) of reaction mixture using calibrated pipette.
	k) Improper mixing of HbA1c dried conjugate, Assay buffer & sample.	Proper mixing must be done using iVortexer.
2. Unexpected high test results	a) High amount of whole blood sample used.	Use appropriate volume of fresh Whole blood sample using calibrated pipette.
	b) Less amount of Assay Buffer used	Use appropriate volume (200 μl) of detection Buffer using calibrated pipette.
	c) Results read beyond 15 minutes	Read the results at 15 minutes.
	d) Improper i.e, high volume of reaction mixture applied to sample well of cartridge	Use appropriate volume of reaction mixture using calibrated pipette.

REFERENCES

1. Segun Adeoye, Sherly Abraham et al.
Anaemia and hemoglobin A1C level. BJMP 2014; 7(1): a706
2. Fatih Ozcelik, Omer Yiginer et al.
Comparison of three methods for measurement of HbA1c. Turk J Biochem 2010; 35(4); 344-349.
3. Jovanovic L, Peterson CM. The clinical utility of glycosylated hemoglobin. AmJ Med 1981; 70: 331-8.
4. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM. Test of glycemia in diabetes. Diabetes Care 1995; 18:896-909.

For in-vitro diagnostic use only, not for medicinal use

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