Spectrum For Diagnostic Industries

HbA1c plus

REF: 386 01 012 **50 Tests**
R1: 1 x 11.5 ml
R2: 1 x 3.8 ml
LR: 2 x 25 ml
C : 1 x 0.5 ml

Intended Use

For the quantitative determination of HbA1c on semi-automated chemistry analyzers.

Background

The glycemic control in diabetes mellitus is performed mainly by the determination of Glucose, but also through quantitative determination of Hemoglobin A1c (HbA1c) in human blood. HbA1c is an indication for the actual glucose levels over the preceding 3 months. It was shown that HbA1c in diabetic subjects can be elevated 2-3 fold over normal and on the other hand approaches normal values when they are under metabolic control.

Test Principle

This method utilizes the interaction of antigen and antibody to determine the HbA1c in whole EDTA blood. HbA1c in test samples is adsorbed onto the surface of latex particles, which react with Anti-HbA1c (antigen-antibody reaction) and gives agglutination.
The amount of agglutination is measured as absorbance which is proportional to HbA1c value.

Reagents

(R1) Latex. Sodium azide (0.95 g/L).

(R2) Anti-human hemoglobin A1c mouse monoclonal antibody and stabilizers.

(C) Calibrator. **Nominal Value stated on the vial Label.**

(LR) Lysing Reagent.

Storage and Stability

Store all reagents refrigerated at 2-8°C. Unopened reagents are stable up to the expiration date printed on the labels.
Opened vials are stable for one month

Specimen Collection and Preparation

Collect venous blood with EDTA using aseptic technique. To determine HbA1c, a hemolysate must be prepared for each sample
1. Dispense **1 ml** of **Lysing Reagent** into patient tubes.
2. Place **10µl** of well mixed whole blood into the Appropriately labeled lysing reagent tube. Mix thoroughly.
3. Allow to stand for **5 minutes** store up to 10 days 2-8°C.

Precautions

1. The reagent is for in vitro diagnostic use only.
2. Reagents are liquid stable, ready-to-use reagents.
3. Mix by inverting at least 10 times before use.
4. Do not mix reagents of different lots.
5. **DO NOT FREEZE**.
6. All human specimens should be regarded as potentially bio hazardous. Therefore, universal precautions should be Used in specimen handling (gloves, lab garments, avoid aerosol production, etc.)

Procedure

Wavelength	630 nm (620 optional)
Method	fixed rate
Temperature	37 °C

	Calibrator	Sample
R1 (µL)	225	225
Control (µL)	15	-
Sample (µL)	-	15
Mix and incubate for 5 min at 37°C.		
R2(µL)	75	75

Mix then read absorbance (**A1**) after 10 seconds, at 630 nm. After 5 minutes, read (**A2**) and calculate ΔA.

Calculation

ΔA (sample or calibrator) = A2 – A1

$$\frac{(\Delta A)\ \text{Sample}}{(\Delta A)\ \text{Calibrator}} \times \text{Calibrator nominal Value} = X$$

The HbA1c concentration can be determined from the supplied table.

N.B : The supplied table is Lot dependant.

Conversion from HbA1c % to mmol/mol

HbA1c %	HbA1C mmol/mol
6.0	42
6.5	48
7.0	53
7.5	59
8.0	65
9.0	75
10	86

Expected Values

(%) DCCT / NGSP
4.0 – 6.0 Non Diabetic
6.0 – 6.5 Mean
6.5 – 8.0 Good Control

Limitations

1. Results may be inconsistent in patients e.g. with opiate addiction, lead-poisoning, alcoholism, ingestion of large doses of aspirin.
2. Elevated levels (> 10%) of HbF may lead to underestimation of HA1c.
3. Hemoglobin variants HbS, HbC and HbE do not interfere in this assay.
4. There is also no interference by labile intermediates, and uremia does not interfere too.

Performance Characteristics

Dynamic Range:
The Hemoglobin A1c assay range is 3.0% to 16.0%.
Results in this range can be reported and used directly.
Linearity: up to 15 %
Correlation:
A study using 40 human specimens between this HA1c procedure and the reference method yielded a correlation coefficient of 0.9874 and a linear regression equation of y = 1.021 x + 0.014
Precision:

Within Run: The intra assay precision was established by assaying blood with two Hemoglobin A1c levels twenty times each.

Level	Mean	% C.V.
Medium	5.7	1.0
High	10.3	0.7

Interferences

1. Bilirubin to 15mg/dL, ascorbic acid to 10mg/dL, triglycerides to 3000mg/dL, Glucose to 4000mg/dL, carbamylated Hb to 5mmol/L and acetylated Hb to 5.0mmol/L do not interfere in the assay.
2. It has been reported that results may be inconsistent in patients who have the conditions like opiate addiction, lead-poisoning, alcoholism, ingestion of large doses of aspirin.

References

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