AutoPure®

Sphera System Packs

HbA1c Direct

Introduction

1. AutoPure HbA1c is a reagent kit for direct quantitative determination of haemoglobin A1c (HbA1c) in whole blood.
2. AutoPure HbA1c is a ready-to-use, two liquid reagent system.
3. With AutoPure HbA1c, the assay is linear upto 16 % (NGSP).

Principle

AutoPure HbA1c assay is based on antigen – antibody interaction to directly determine the HbA1c concentration in whole blood. Total haemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added, latex-HbA1c-mouse antihuman HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse IgG polyclonal antibody interacts with the monoclonal antibody. The amount of agglutination is proportional to the concentration of HbA1c in the specimen and is measured photometrically.

Reagent Storage, Stability & Handling

AutoPure HbA1c is a ready-to-use, two liquid reagent system.

Shelf life
Stable till expiry date indicated on the label when stored at 2°-8°C.

On – Board Reagent Stability
R1: 4 weeks at 2°-8°C after opening.
R2: 4 weeks at 2°-8°C after opening.
Protect the reagent from light and contamination.

Do not freeze the reagent.

Components & Concentration of Working Solution

Component	Concentration
R1	
Latex	0.13 %
R2	
Mouse anti-human HbA1c monoclonal antibody	> 0.04 mg/ml
Goat anti-Mouse IgG polyclonal antibody	> 0.06 mg/dl
R3	
Haemolysis reagent	

Specimen Collection & Preservation

Collect sample using standard sampling tube. Whole blood with EDTA is the specimen of choice. HbA1c in whole blood collected with EDTA is stable for one week at 2°-8°C.

To determine HbA1c, a haemolysate must be prepared for each sample as follows:
1. Dispense 0.5 ml Haemolysis reagent into appropriately labeled tube.
2. Add 10 µl of well mixed whole blood.
3. Mix well and allow to stand for 5 minutes or until complete lysis is evident.
(AutoPure HbA1c calibrators and AutoPure HbA1c Controls should also be treated for haemolysate preparation in the same manner.)

Procedure

AutoPure HbA1c can be used on various automated analyzers. General procedure is as described below.

PROCEDURE FOR SEMI-AUTOMATED ANALYZERS

Reaction Type	End Point
Reaction direction	Increasing
Wavelength	650 nm.
Flowcell temperature	37°C
Zero setting with	Reagent Blank
Sample volume	10 µl
Reagent 1 (R1) volume	360 µl
Reagent 2 (R2) volume	120 µl
Linearity	16 %

Manual assay procedure:
Perform the assay as given below:

	Blank	Calibrator	Sample
R1	**360 µl**	**360 µl**	**360 µl**
Haemolysate	**-**	**10 µl**	**10 µl**
Mix and incubate for 5 minutes at 37°C.			
R2	**120 µl**	**120 µl**	**120 µl**

Mix and incubate for 5 minutes at 37°C. Measure the absorbance of assay mixture against the reagent blank at 650 nm.
Calculation :
% HbA1c in sample is calculated using the calibration curve generated by plotting absorbance versus concentration of each calibrator.

Calculations

Fully automated systems automatically calculate the HbA1c concentration of each sample.

Application Sheet

For system applications, contact our local Accurex representative.

Calibration

For calibration, it is recommended to use AutoPure HbA1c calibrator from Accurex.

Generate 5 point non-linear calibration curve .(Use saline as first calibrator with concentration '0') . Other commercially available HbA1c calibrators have not been tested with this assay and may not be supported by AutoPure HbA1c. Refer to the AutoPure HbA1c calibrator kit package insert for a description of assignment procedures and instructions.

Calibration frequency
Re - calibration is recommended

- Whenever the reagent lot is changed.
- As per the requirement of quality control procedures.

Quality Control

Each batch of AutoPure HbA1c is assayed with multiple quality control material prior to release.
To ensure adequate quality control, it is recommended that the laboratory should use a normal and an abnormal commercial reference control material. It should be realized that the use of quality control material checks both reagent and the instrument functions together. The value of these controls should fall within the specified limits. If control values fall outside specified limits, each of the below criteria should be cross-checked and corrected:

- Proper instrument function – wavelength setting, light source and temperature control.
- Cleanliness of probes and cuvettes.
- Bacterial contamination of wash water used by the instrument.
- Expiry date of the reagent kit.

Expected Values

According to NGSP:
Non-diabetic : Less than 6 %
Good Control : Less than 7 %

Note:

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own reference range. For diagnostic purposes, the HbA1c results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Performance Characteristics

Linearity

With AutoPure HbA1c, the assay is linear upto 16 % (NGSP). Determine samples with higher concentrations via the rerun function.

Interference

There is no significant interference from samples containing upto 7.5 mmol/L of carbamylated Hb, 5.0 mmol/L of acetylated Hb, 50 mg/dl of bilirubin, 50 mg/dl of ascorbic acid and 2000 mg/dl of triglycerides. Labile intermediates (Schiff base) and Haemoglobin variants (HbA2, HbC & HbS) do not interfere with this assay. Elevated levels of HbF may lead to underestimation of HbA1c. Results may be inconsistent in patients with conditions of opiate addiction, lead poisoning, alcoholism and large dose of aspirin ingestion.

Precision

Reproducibility was determined using two levels of sample as shown below:

	Within run			Between run		
Sample	Mean %	SD %	%CV	Mean %	SD %	%CV
Low	5.48	0.078	1.43	5.48	0.152	2.77
High	10.28	0.176	1.72	10.28	0.275	2.68

Co - Relation Studies

A comparison of HbA1c determination using AutoPure HbA1c and an automated HPLC method gave the following co-relation (%):
Linear Regression
y =1.050x – 0.481
r = 0.988
Sy.x = 0.332
No. of samples measured : 40

References

1. Gonen, B., and Rubenstein, A. H., ***Diabetologia*** 15, 1 (1978).
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3. Engbaek, F., et al, ***Clin. Chem.*** 35, p. 93-97 (1989).
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6. Ceriello, A., et al, ***Diabetologia*** 22, p. 379 (1982).
7. Fluckiger, R., et al, ***New Eng. J. Med.*** 304, p. 823-827 (1981).
8. Trivelli, L.A., Ranney, H. M., and Lai, H.T., ***New Eng. J. Med.*** 284, 353 (1971).
9. American Diabetes Association: Clinical Practice Recommendations (Position Statement). ***Diabetes Care 24*** (Suppl.1): S33-S55 (2001).

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ISO 13485, ISO 9001 CERTIFIED COMPANY

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HbA1c Protocol

It is a two step process –

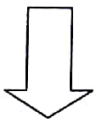
1. Sample Pre-treatment
2. Test Procedure

Sample Pre-treatment for FAA & SAA

R_3 – 500 µl (Haemolysing Agent)

+

10 µl (**EDTA** Whole blood/Calibrator)



Keep at **Room Temp** for **5 – 10** mins

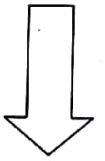
Haemolysate Ready

Test Procedure for SAA

R_1 – 360 µl

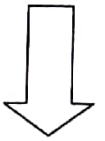
+

10 µl Haemolysate



Incubate for 5 mins @ 37^{0c}

R_2 – 120 µl



Incubate for 5 mins @ 37^{0c}

Aspirate @ 630 – 670 nm

HbA1c Protocol

Note – Very Imp

The **Sample Pre-treatment** will be common for both **Semi & Fully** Automated instruments.

1. On Sphera use **AutoPure system Pack only.**
2. On FAA - Process the sample in **separate/in individual** or **last in a batch,** do not run **in-between** any chemistry for better results.
3. **Shake/Mix R1** well before process, if kept for a longer time on board.

Calibration On Sphera

1. Follow the program sheet & enter the particulars as given.
2. Enter 4 no. of Calibrators with the values in lot management as **A1c1,A1c2,A1c3 & A1c4.**
3. In **Methods i.e in** calibration chart/Designations select **1st as BLANK** from drop down followed by **4 calibrators – The BLANK** selected is actually the **Diluent** at the 30th position, so while executing **the test/calibration no** need to assign the position for **BLANK onboard.**
4. **Blank correction** to be selected, a MUST.
5. The given calibrators, in-case of unopened pack - to be first reconstituted with **500µl of DW &** let it stand for 15mins @ Room Temperature, after which follow the Haemolysing step i.e Sample Pre-treatment as mentioned above.
6. Interpolation - Polylinear