

Intended Use

For the quantitative determination of Hemoglobin A1c (HbA1c) in human blood using the Yumizen C230 and Yumizen C240 analyzers. The determination of HbA1c is most commonly performed for the evaluation of glycemic control in diabetes mellitus. HbA1c values provide an indication of glucose levels over the preceding 4-8 weeks. A higher HbA1c value indicates poorer glycemic control. For *in vitro* diagnostic use only. **Rx Only.**

Summary and Explanation of Test

Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. In a classical study, Trivelli et al¹ showed Hemoglobin A1c in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that Hemoglobin A1c serve as an indicator of metabolic control of the diabetic, since Hemoglobin A1c levels approach normal values for diabetics in metabolic control.^{2,3,4}

Hemoglobin A1c has been defined operationally as the "fast fraction" hemoglobins (HbA_{1a}, A_{1b}, A_{1c}) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA₀. The present procedure utilizes an antigen and antibody reaction to directly determine the concentration of the HbA1c.

Principle

This method utilizes the interaction of antigen and antibody to directly determine the HbA1c in whole blood. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse antihuman HbA1c monoclonal antibody is added (R2), latex-HbA1c-mouse anti human 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 amount of HbA1c absorbed on to the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

Reagents

R1: Latex 0.13%, Buffer, stabilizer.

R2: Mouse anti-human HbA1c monoclonal antibody 0.05mg/ml, goat anti-mouse IgG polyclonal antibody 0.08mg/dl, Buffer, stabilizers.

Hemolysis reagent: water and stabilizers.

Reagent Storage

Store all reagents refrigerated at 2-8°C.

Reagent Preparation

R1, R2 and Hemolysis reagents are supplied as ready to use liquids. Mix gently before use.

Reagent Deterioration

Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

Refer to specific instrument application for suggested settings.

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Not for internal or external use in humans or animals.

Specimen Collection and Preparation

Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants are required. Collect venous blood with EDTA using aseptic technique. All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.).

To determine HbA1c, a hemolysate must be prepared for each sample:

1. Dispense 1ml Hemolysis Reagent into tubes labeled: Control, Patients, etc. Note: Plastic or glass tubes of appropriate size are acceptable.
2. Place 20ul of well mixed whole blood into the appropriately labeled lyse reagent tube. Mix.
3. Allow to stand for 5 minutes or until complete lysis is evident. Hemolysates may be stored up to 10 days at 2-8°C.

Storage and Stability

1. All reagents are stable to the expiration date stated on the labels. Do not use the reagents past their expiration date.
2. R1 and R2 are stable for at least one month after opening stored at 2-8°C.
3. Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8°C.⁵

Interferences

1. Bilirubin to 50mg/dL, ascorbic acid to 50mg/dL, triglycerides to 2000mg/dL, carbamylated Hb to 7.5mmol/L and acetylated Hb to 5.0mmol/L do not interfere in this assay.
2. It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.^{6, 7, 8, 9}
3. It has been reported that elevated levels of HbF may lead to underestimation of HA1c.¹⁰ Also, it has been reported that labile intermediates (Schiff base) are not detected and do not interfere with HbA1c determination by immunoassay.⁵
4. It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
5. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

Materials Provided

Refer to "Reagents"

Materials Required but not Provided

1. Yumizen C230 / Yumizen C240 Analyzer.
2. Yumizen C230 / Yumizen C240 Operation manual.
3. Pipettes to dispense 20 µl and 1 ml and Test Tubes to hold 1.02 ml.
4. Pointe Hemoglobin A1c calibrator set and control set.

Test Parameters*

Test:	HbA1c	Chemistry:	HbA1c
Chemistry No.:	219	Print Name:	HbA1c
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	670 nm	Sec. Wave:	
Decimal.:	0.1	Samp. Type:	Serum
Blank Time:	0 0	Reaction Time:	33 34
Unit:	%A1c	Incubation Time:	8

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard;	4 uL	4 uL	200 uL	150 uL	uL
Decreased;	uL	uL	uL	50	
Increased;	uL	uL	uL		

Linearity Range (Standard):	2-16	Linearity Limit:	
Linearity Range (Decreased):		Substrate Depletion:	
Linearity Range (Increased):		Mixed Blank Abs.:	-40000 40000
R1 Blank Abs.:	-40000 40000	On-board Stability:	30 Day (s)
Blank Response	-40000 40000	Reagent Alarm Limit:	10
Twin Chemistry:			

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:	
Range:	Flag:

***YC240 Only**

Pointe Hemoglobin A1c Reagent Set

Slope Offset:	Slope	Offset	Unit
	1	0	%

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:			
Sample Type:	Gender:	Age Range:	Ref. Range: Critical Range: Unit:

Calibration Setup Parameters

Chem:	A1C	
Calibration Setting		
Math Model:	Spline	
Factor:	Replicates: 1	
Acceptance Limits		
Cal Time:	168 hr.	
Slope Diff:	SD:	
Sensitivity:		
Repeatability:		
Deter Coeff.:	* User Defined	
Auto Calib.	<input type="checkbox"/> Cal Time	

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
A1c 1	*	*	*
A1c 2	*	*	*
A1c 3	*	*	*
A1c 4	*	*	*

Limitations

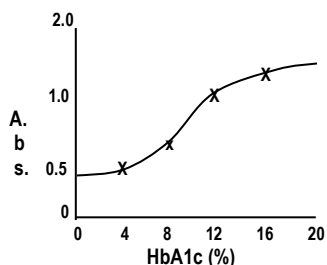
- This assay should not be used for the diagnosis of diabetes mellitus.
- Patient specimens should always be assayed using a calibration curve.
- It has been reported that results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, alcoholism, ingest large doses of aspirin.^{6, 7, 8, 9}
- It has been reported that elevated levels of HbF may lead to underestimation of HA1c and, that uremia does not interfere with HbA1c determination by immunoassay.¹⁰ It has been reported that labile intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunoassay.⁵
- It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
- Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

Quality Control

The reliability of test results should be monitored whenever patient samples are assayed using a standard and quality control materials analyzed in the same manner employed for the unknowns. We suggest the use of commercially available Hemoglobin A1c controls with an assayed range. If controls do not fall into the assayed range patient values from that run should not be reported. The run should be repeated, making sure that all mixing and handling instructions are strictly followed. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements. Linearity of the assay should be verified with a commercial linearity check set, or dilutions of a high specimen, at least every six months.

Calculations / Results

HbA1c results for the unknowns and controls are determined using the prepared calibration curve. An example curve is illustrated below.



Expected Values¹¹

Recommended Values: less than 6% for a non-diabetic, less than 7% for glycemic control of a person with diabetes.

Each laboratory should establish its own expected values. In using Hemoglobin A1c to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a 3-4 week time lag before Hemoglobin A1c reflects changes in blood glucose level.

Performance

- Linearity:** The Hemoglobin A1c assay range is 2.0%-16.0%.
- Comparison:** A study using 33 human specimens assayed using this Hemoglobin A1c procedure on the Yumizen 200 series analyzers and a similar analyzer, yielded a correlation coefficient of 0.983 and a linear regression equation of $y=0.973x + 0.24$.
- Precision:** Precision studies were performed using the Yumizen 200 series analyzers following a modification of the guidelines which are contained in NCCLS document EP5-T2.¹²

Within Run:

Level	Mean	Std. Dev.	% C.V.
Low	5.03	0.05	1.00
High	11.62	0.17	1.50

Day to Day: The between day precision was established by assaying two blood samples following NCCLS protocol EP5 on a Hitachi 917.

Level	Mean	Std. Dev.	% C.V.
Low	5.48	0.152	2.77
High	10.28	0.275	2.68

*NOTE: Day to Day doesn't reflect Yumizen 200 series analyzers performance.

- Sensitivity:** Sensitivity was investigated by reading the change in absorbance at 660nm for a saline sample and a whole blood sample with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used (Hitachi 717), the HbA1c reagent showed little or no drift on the zero sample. Under the reaction conditions described, a 0.073 absorbance change is approximately equivalent to 1.0% HbA1c.

References

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- American Diabetes Association: Clinical Practice Recommendations (Position Statement). Diabetes Care 24 (Suppl. 1): S33-S55, (2001).
- NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD <i>In vitro</i> diagnostic medical device	Temperature limitation
Consult instructions for use	Rx Only: Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

12-H7546-40	Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188	
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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge. Rev. 11/23 P803-H7546-MIN