Pointe Hemoglobin A1c Reagent Set

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Intended Use

For the quantitative determination of Hemoglobin A1c (HbA1c) in human blood using the Yumizen C560 analyzer. 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 al[1] 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 HbA_0. 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.

Reagent Storage

Store all reagents refrigerated at 2-8°C. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions

Reagent Preparation

R1 and R2 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.

Precautions and Hazards

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

Hazards:

Hazard Classifications: Not a hazardous substance or mixture.
Pictogram and Signal Word: Not required. **Refer to the Safety Data Sheet for this product (SDS-H7546) available by calling 1-734-487-8300.**
Hazard & Precautionary Statement: Not a hazardous substance or mixture.

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 is prepared by the analyzer using the on-board lyse application:

1. Well mixed whole blood is placed directly onto sample carousel or into sample cups. An inaccurate result could be produced from an unmixed whole blood sample. Note: If plastic or glass tubes are used, verify appropriate size.
2. Erythrocytes in a whole blood sample will settle out over time. Analysis of sample should be initiated as soon as possible after samples have been placed onto analyzer.

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. R1 and R2 are stable for at least one month after opening stored at 2-8°C or placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.
2. 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.

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Materials Provided
Refer to "Reagents"

Materials Required but not Provided
1. Yumizen C560 Analyzer.
2. Yumizen C560 Operation manual.
3. Pipettes
4. Hemoglobin A1c calibrator set (Catalog Number: H7541-CAL) and control set (Catalog Number: H7541-CTL).

Limitations
1. This assay should not be used for the diagnosis of diabetes mellitus.
2. Patient specimens should always be assayed using a calibration curve.
3. 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]
4. 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]
5. It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
6. 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.

Expected Values[11]
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
1. Assay Range: The Hemoglobin A1c assay range is 2.0%-16.0%.
2. Correlation: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	HbA1c
N	40
Mean HbA1c (%)	6.949
Range (%)	4.8-10.0
Standard Deviation	1.478
Regression Analysis	y = 0.934x + 0.302
Correlation Coefficient	0.9889

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[12]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	5.96	8.32	11.66
Standard Deviation	0.05	0.04	0.05
Coefficient of Variation (%)	0.8%	0.5%	0.4%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	6.00	8.33	11.47
Standard Deviation	0.04	0.06	0.10
Coefficient of Variation (%)	0.6%	0.7%	0.8%

4. Sensitivity**:** 2SD limit of detection (95% Conf) = 0.2%

References

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2. Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978).
3. Gabbay, K.H., Hasty, K., Breslow, J.L., Ellison, R.C., Bunn, H.F., and Gallop, P.M., J. Clin. Endocrinol. Metab. 44, 859 (1977).
4. Bates, H.M., Lab. Mang., Vol 16 (Jan. 1978).
5. Tietz, N.W., Textbook of Clinical Chemistry, Philadelphia, W.B. Saunders Company, p.794-795 (1999).
6. Ceriello, A., et al, Diabetologia 22, p. 379 (1982).
7. Little, R.R., et al, Clin. Chem. 32, pp. 358-360 (1986).
8. Fluckiger, R., et al, New Eng.J. Med. 304 pp. 823-827 (1981).
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11. American Diabetes Association: Clinical Practice Recommendations (Position Statement). Diabetes Care 24 (Suppl. 1): S33-S55, (2001).
12. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

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CHEMISTRY PARAMETERS

Chem:	HbA1c	No.:	219	Sample Type:	Whole Blood
Chemistry:	Hemoglobin A1c			Print Name:	HbA1c
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	660			Sec Wave:	
Unit:	%			Decimal	0.1
Blank Time:	0 0			Reaction Time:	80 82

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	4.0 ul	4.0 ul	200 ul	R1: 150 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: 50 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☒ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	2	16	Linearity Limit:	
Linearity Range (Decreased)	____	____	Substrate Depletion:	
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator: * Lot No.: *

Exp Date: *

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	HbA1c	*	%
HbA1c Cal 1	*	*	*	HbA1c	*	%
HbA1c Cal 2	*	*	*	HbA1c	*	%
HbA1c Cal 3	*	*	*	HbA1c	*	%
HbA1c Cal 4	*	*	*	HbA1c	*	%

Calibration Setup

Chem: HbA1c

Calibration Settings

Math Model: Spline

Factor: Replicates: 1

Acceptance Limits

Cal Time: 168 Hour

Slope Diff: --- SD: ---

Sensitivity : --- Repeatability: ---

Deter Coeff: ---

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.

* Indicates user defined parameter.

REF 14-H7546-160

Manufactured by HORIBA Instruments Incorporated-Pointe Brand
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2°C 8°C IVD

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.

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CE

Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number

Manufacturer Temperature limitation Consult instructions for use

IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only

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