Hemoglobin A1c Reagent Set

Intended Use
For the quantitative determination of Hemoglobin A1c (HbA1c) in human blood. 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.

Summary and Explanation of Test
Throughout the circulatory life of the red cell, Hemoglobin A1c is formed continuously by the addition 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 (HbA1a, A1b, A1c) 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 anti-human 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 reacts 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. (Included in 40ml kit, not 120ml kit)

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.

Materials Provided
Refer to “Reagents”

Materials Required but not Provided
1. Pipettes to dispense 20 ul and 1 ml and Test Tubes to hold 1.02 ml.
2. Hemoglobin A1c calibrator set, control set, and Hemolysis rgt for 120 ml kit.

Procedure (automated-Hitachi 717)

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>HbA1c</th>
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</thead>
<tbody>
<tr>
<td>ASSAY CODE</td>
<td>[1-POINT]:[50]-[0]</td>
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<tr>
<td>SAMPLE VOLUME</td>
<td>[5] [3]</td>
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<tr>
<td>R1 VOLUME</td>
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<td>R2 VOLUME</td>
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<tr>
<td>WAVELENGTH</td>
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<td>STD (3) CONC-POS</td>
<td>[**] [3]</td>
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<tr>
<td>STD (4) CONC-POS</td>
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<td>STD (5) CONC-POS</td>
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<tr>
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<tr>
<td>SENSITIVITY LIMIT</td>
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<tr>
<td>ABS LIMIT (INC/DEC)</td>
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</table>
Hemoglobin A1c
Reagent Set

PROZONE LIMIT [1.0-3.0]
EXPECTED VALUE [1.0-1.4]
PANIC VALUE [2.5-9.0]
INSTRUMENT FACTOR [1.0]

* Use Saline for the 0.0 Calibrator
** Input the values of the calibrator set being used

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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 HbA1c 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.

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
1. Linearity: The Hemoglobin A1c assay range is 2.0%-16.0%.

Comparison: A study using 40 human specimens between this Hemoglobin A1c procedure and an automated HPLC procedure (Tosoh) yielded a correlation coefficient of 0.988 and a linear regression equation of y=0.983x +0.140. (Syx = 0.230)

Precision:
Within Run: The within run precision was established by assaying blood samples following NCCLS protocol EP5 on a Hitachi 717.

Day to Day: The between day precision was established by assaying blood samples following NCCLS protocol EPS on a Hitachi 717.

References