**HbA1c Glycated Hemoglobin**  
*Latex Turbidimetry*

### Clinical Significance
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 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.

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 a 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 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

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Components</th>
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<tr>
<td>R1</td>
<td>Latex 0.13%, Buffer, stabilizer.</td>
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<tr>
<td>R2(When combined)</td>
<td>Mouse anti-human HbA1c monoclonal antibody 0.05mg/ml, goat anti-mouse IgG polyclonal antibody 0.08mg/ml, Buffer, stabilizers.</td>
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<tr>
<td>R3(Hemolysis agent)</td>
<td>Water and stabilizers</td>
</tr>
<tr>
<td>Optional</td>
<td>HbAB1c CAL., HbAB1c (4) Calibrators, HbAB1c CONTROL., HbAB1c (2) Controls.</td>
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</tbody>
</table>

### Precautions
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

### Preparation
R1 and R3 are ready to use. R2 (combined): transfer the content of one bottle R2b to one bottle R2a. Homogenize the working reagent avoiding foam formation. R2 when combined is stable during 4 weeks at 2-8°C.

### Storage and Stability
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations prevented during their use. Do not use reagents over the expiration date. R1 and R2 are stable for at least one month after opening stored at 2-8°C. Hemoglobin A1c in whole blood collected with EDTA is stable for one week at 2-8°C.

### Reagent deterioration
Presence of particles and turbidity.

### Materials Required but not Provided
- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 540 nm filter.

### Specimen
Collect venous blood with EDTA using aseptic technique. To determine HbA1c, a hemolysate must be prepared for each sample:

1. Dispense 1 mL of R3 (Hemolysis Reagent) into tubes labeled: Calibrator, Control, Patients, etc. Note: Plastic or glass tubes of appropriate size are acceptable.
2. Place 20 µL 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.

### Assay Procedure for Multi-point:

1. Bring the R1 and R2(combined) reagent and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   - Wavelength: 660 nm
   - Temperature: 37°C
   - Cuvette light path: 1 cm
3. Mix and incubate 5 minutes. Then add:
   - R1. Diluent (µL) 360
   - Hemolysate Calibrator or sample(µL) 10
4. Pipette into a cuvette:
   - R2. (Combined) (µL) 120
5. Mix and incubate 5 minutes. Then add:
   - R2. (Combined) (µL) 100
6. Mix and read absorbance after 5 minutes (A) of the R2 addition.

### Euro has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

### Calculations
**HbA1c concentration (%)**
Plot (A) obtained against the HbA1c concentration of each calibrator (1 to 4 Level). HbA1c percentage in the sample is calculated by interpolation of its (A) in the calibration curve.

### Assay Procedure for Single-point:

1. Wavelength: 660 nm
2. Temperature: 37°C
3. Cuvette light path: 1 cm
4. Mix and incubate 5 minutes. Then prepare R2a + R2b in the ratio 20+1 (e.g.:1ml of R2a+ 50µL of R2b)
5. Mix and read absorbance A1 at 5 sec and A2 at 300 sec.

### Calculations:

\[
\text{HbA1c(\%)} = \frac{\Delta A_{\text{sample}} / \text{min}}{\Delta A_{\text{calibrator}} / \text{min}} \times \text{Calibrator value}
\]
Quality Control
Control Sera are recommended to monitor the performance of manual and automated assay procedures. HbA1c Control are available on request.

Reference Values
Less than 6% for a non-diabetic.
Less than 7% for glycemic control of a person with diabetic
Each laboratory should establish its own reference range.

Reagent Performance
Linearity: The Hemoglobin A1c assay range is 2.0%-16.0%.
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. A 0.073 absorbance change is approximately equivalent to 1.0% HbA1c.
Precision: The reagent has been tested using two blood samples (Intra-assay) / control samples (Inter-assay) in an EP5-based study.

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<tr>
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<th>Intra-assay</th>
<th>Inter-assay</th>
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<tbody>
<tr>
<td>Mean (g/dL)</td>
<td>5.48 10.28</td>
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</tr>
<tr>
<td>SD</td>
<td>0.078 0.176</td>
<td>0.152 0.275</td>
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<tr>
<td>CV (%)</td>
<td>1.43 1.72</td>
<td>2.77 2.68</td>
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Correlation: Results obtained using this procedure (y) were compared to those obtained using an automated HPLC procedure (x) with similar characteristics. 40 samples of HbA1B were assayed. The correlation coefficient (r) was 0.988 and the regression equation y = 1.050x -0.481.

The results of the performance characteristics depend on the analyzer used.

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. This assay should not be used as the unique test for the diagnosis of diabetes mellitus. Other tests must be also considered to establish a correct diagnosis.
3. Patient specimens should always be assayed using a calibration curve.
4. 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.
5. 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 immunassay. It has been reported that labile intermediates (Schiff base) are not detected and therefore, do not interfere with HbA1c determination by immunassay.
6. It has been determined that Hemoglobin variants HbA2, HbC and HbS do not interfere with this method.
7. Other very rare variants of hemoglobin (e.g. HbE) have not been assessed.

NOTES
Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Literature