Diagnostic reagent for quantitative in vitro determination of hemoglobin A1c in whole blood on photometric systems

Order Information
Cat. No. Kit size
133299910930 R1 3 x 20 mL + R2 2 x 10 mL + R3 1 x 10 mL
133299910935 R1 2 x 15 mL + R2 1 x 10 mL + R3 1 x 5 mL
13329990380 R1 2 x 15 mL + R2 1 x 10 mL + R3 1 x 5 mL
145709910113 1 x 500 mL oneHbA1c Hemolyzing Solution

Summary [1, 2, 12]
Hemoglobin A1c (HbA1c) is a glycated hemoglobin which is formed by the non-enzymatic reaction of glucose with native hemoglobin. This process runs continuously throughout the circulatory life of the red cell (average life time 100 - 120 days). The rate of glycation is directly proportional to the concentration of glucose in the blood. The blood level of HbA1c represents the average blood glucose level over the preceding 6 to 8 weeks (due to the kinetics of erythrocyte turnover this period is more affected by the blood glucose level than the preceding weeks). Therefore, HbA1c is suitable for retrospective long-term monitoring of blood glucose concentration in individuals with diabetes mellitus. Clinical studies have shown that lowering of HbA1c level can help to prevent or delay the incidence of late diabetic complications. Besides, HbA1c testing may be used for diagnosis of diabetes mellitus. As the amount of HbA1c also depends on the total quantity of hemoglobin the reported HbA1c value is indicated as a percentage of the total hemoglobin concentration. Falsely low values (low HbA1c despite high blood glucose) may occur in people with conditions with shortened red blood cell survival (hemolytic diseases) or significant recent blood loss (higher fraction of young erythrocytes). Falsely high values (high HbA1c despite normal blood glucose) have been reported in iron deficiency anemia (high proportion of old erythrocytes). These circumstances have to be considered in clinical interpretation of HbA1c values.

Method and Principle
Particle enhanced immunoturbidimetric test
HbA1c is determined directly without measurement of total hemoglobin. Total Hb and HbA1c in hemolyzed blood bind with the same affinity to particles in R1. The amount of binding is proportional to the relative concentration of both substances in the blood. Mouse anti-human HbA1c monoclonal antibody (R2) binds to particle bound HbA1c. Goat anti-mouse IgG polyclonal antibody (R3) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the relative concentration of both substances in the blood. The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and protected from light.

HbA1c and Average Glucose Concentrations [10]
Due to a linear correlation between hemoglobin A1c and average glucose concentrations HbA1c values can be converted in estimated average glucose values by means of the following equations:

Standardization according to IFCC (calculated referring to literature reference 10):
Average glucose conc. [mg/dL] = 2.63 x HbA1c% + 15.01
Average glucose conc. [mmol/L] = 0.146 x HbA1c% + 0.829
a: HbA1c values in % NGSP
b: HbA1c values in % IFCC

Storage Instructions and Reagent Stability
The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C.

Warnings and Precautions
1. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
2. In very rare cases, samples of patients with gammopathy might give falsified results [13].
3. Heterophile antibodies in patient samples may cause falsified results.
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
5. Immediately after HbA1c measurement cleaning of cuvettes is necessary. Use the alkaline cuvette washing solution which is recommended by the analyzer manufacturer.
6. For professional use only!

Waste Management
Please refer to local legal requirements.

Materials Required
General laboratory equipment

Specimen
Whole blood collected with EDTA
Discard contaminated specimens.
Please collect whole blood by standard venipuncture and fill the blood collection tube according to manufacturer specifications.

Sample Preparation:
For sample preparation the DiaSys oneHbA1c Hemolyzing Solution Cat. No 145709910113 is required.

Sample preparation:
Hemolyzing Solution
1000 µL
Sample/Calibrator/Control
20 µL
Mix and allow to stand for 5 minutes or until complete lysis is apparent.

Specimen Stability [7]:
Whole blood
1 week at 2 – 8°C
Hemolysate
10 hours at 15 – 25°C
Hemolysate
10 days at 2 – 8°C

Assay Procedure
Application sheets for automated systems are available on request.

Wavelength
660 nm
Optical path
1 cm
Temperature
37°C
Measurement
Against air
**Sensitivity/Limit of Detection**

The limit of detection (LOQ) is 30 mmol/mol HbA1c according to IFCC (4.9% HbA1c according to DCCT/NGSP).

**Precision**

(Hitachi 917. 3-component system; Values according to IFCC)

<table>
<thead>
<tr>
<th>Within-run precision</th>
<th>Mean [mmol/mol]</th>
<th>SD [mmol/mol]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>34.1</td>
<td>0.541</td>
<td>1.59</td>
</tr>
<tr>
<td>Sample 2</td>
<td>57.1</td>
<td>0.655</td>
<td>0.973</td>
</tr>
<tr>
<td>Sample 3</td>
<td>84.7</td>
<td>1.02</td>
<td>1.21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between day precision</th>
<th>Mean [mmol/mol]</th>
<th>SD [mmol/mol]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>37.6</td>
<td>0.771</td>
<td>2.05</td>
</tr>
<tr>
<td>Sample 2</td>
<td>54.3</td>
<td>0.772</td>
<td>1.42</td>
</tr>
<tr>
<td>Sample 3</td>
<td>85.3</td>
<td>1.50</td>
<td>1.76</td>
</tr>
</tbody>
</table>

**Total precision**

<table>
<thead>
<tr>
<th>CLSI</th>
<th>Mean [mmol/mol]</th>
<th>SD [mmol/mol]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>37.6</td>
<td>0.776</td>
<td>2.06</td>
</tr>
<tr>
<td>Sample 2</td>
<td>54.7</td>
<td>0.953</td>
<td>1.74</td>
</tr>
<tr>
<td>Sample 3</td>
<td>85.7</td>
<td>1.62</td>
<td>1.89</td>
</tr>
</tbody>
</table>

**Method Comparison**

A comparison of DiaSys oneHbA1c FS (y) to a commercially available assay (x) using 88 samples gave following results (IFCC values): y = 1.05 x – 2.94 mmol/mol; r = 0.997.

A comparison of DiaSys oneHbA1c FS (y) to a HPLC assay (x) using 100 samples gave following results (IFCC values): y = 1.04 x – 1.75 mmol/mol; r = 0.997.

**Reference Range**

Suggested target values for HbA1c [8]:

- Non-diabetics (≥ 20 – 42 mmol/mol (IFCC)
- Target of therapy: < 5.7 % (NGSP)
- Change of therapy: > 64

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

**HbA1c cut point value for diagnosis of diabetes mellitus [12]:**

According to a recommendation of the American Diabetes Association (ADA): ≥ 6.5% (NGSP) (48 mmol/mol (IFCC)). Patents with HbA1c values in the range of 5.7 – 6.4% HbA1c (NGSP) or 39 – 46 mmol/mol HbA1c (IFCC) may be at high risk of developing diabetes.

**Literature**

7. Data on file at DiaSys Diagnostic Systems GmbH.