HEMOGLOBIN A1c. Particle enhanced immuno-turbidimetric test. HbA1c is determined directly without measurement of total haemoglobin.

METHOD AND PRINCIPLE
Haemoglobin A1c (HbA1c) is a glycated hemoglobin which is formed by the non-enzymatic reaction of glucose with native haemoglobin. 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.

As the amount of HbA1c also depends on the total quantity of haemoglobin the reported HbA1c value is indicated as a percentage of the total haemoglobin 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.

• Sample and addition of R1 (latex reagent)
• Addition of R2 (anti-HbA1c reagent) and start of reaction

This method is based on latex agglutination. HbA1c in the test samples is absorbed onto the surface of latex particles, which react with anti-HbA1c (antigen-antibody reaction). The turbidity caused by latex agglutination is measured at 660 nm and the HbA1c concentration in whole blood is calculated from the standard curve.

PRECAUTIONS / DANGER SYMBOLS
The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

REAGENT PREPARATION AND STABILITY
For sample preparation is required distilled water.
Distilled water 500 μL
Sample 10 μL
Mix and allow to stand for 5 minutes or until complete lysis is apparent. Note: Calibrators and controls are ready to use.
R1: Ready for use. 30 mL
R2: Ready for use. 10 mL

Unopened kit components: Up to the expiration date at +2°C to +8°C
Onboard stability at +2°C to +8°C: R1: 30 days
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.

PROCEDURE
Wavelength: 660 nm
Cuvette: 1 cm
Temperature: +37°C
Measurement: against reagent blank
Reaction: Fixed Time
Calibration: Multipoint

Agitate and incubate for 2 minutes

Agitate and transfer to cuvette. After incubation for 20 seconds at 37°C read absorbance of calibrators and sample at 0 sec. and then 220 sec. Calculate ΔE/min. at 660 nm.

EXPECTED VALUES
Reference intervals should be established or verified by the laboratory based on an appropriate non-diabetic patient population.

Suggested target values for HbA1c:

<table>
<thead>
<tr>
<th>%NGSP</th>
<th>mmol/mol</th>
<th>%IFCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diabetics</td>
<td>4-6</td>
<td>20.22 - 42.08</td>
</tr>
<tr>
<td>Target of Therapy</td>
<td>&lt;7</td>
<td>&lt;53.01</td>
</tr>
<tr>
<td>Change Therapy</td>
<td>&gt;8</td>
<td>&gt;83.94</td>
</tr>
</tbody>
</table>

STORAGE INSTRUCTIONS AND REAGENT STABILITY

Specimen stability:
Whole blood 1 week at 2 – 8 °C
Hemolysate 10 hours at 15 - 25 °C
Hemolysate 10 days at 2 – 8 °C

REAGENT COMPOSITION

R1: Latex
Buffer 25mmol/L
Stabilizer 1.5%
Stabilizer 0.95 g/L
R2: Buffer 15mmol/L
Anti-HbA1c mouse monoclonal antibody 5.6 mg/dL
Anti-mouse IgG antibody 12 mg/dL
Stabilizer 0.95 g/L

SPECIMEN
Whole blood collected with EDTA. Discard contaminated specimens.

Calibrators (1-5)

<table>
<thead>
<tr>
<th>Reagent 1</th>
<th>White</th>
<th>Sample</th>
<th>Calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>8 μL</td>
<td>300 μL</td>
<td>300 μL</td>
</tr>
<tr>
<td>Sample</td>
<td>8 μL</td>
<td>100 μL</td>
<td>100 μL</td>
</tr>
</tbody>
</table>

Calculation:

ΔE = E0 - E220

Agitate and incubate for 2 minutes

Agitate and transfer to cuvette. After incubation for 20 seconds at 37°C read absorbance of calibrators and sample at 0 sec. and then 220 sec. Calculate ΔE/min. at 660 nm.
WASTE DISPOSAL
The disposal of the product must be in accordance with local regulation concerning waste disposal.

QUALITY CONTROL
The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Use MTD Diagnostics Controls:
- TUC1010 HbA1c Control Set. 2 Levels

CALIBRATION
Results will depend on the accuracy of the instrument calibration, assay settings, the reagent/specimen ratio and the temperature control.

The concentration of HbA1c in unknown samples is derived from a calibration curve using an appropriate mathematical model such as spline. The calibration curve is obtained with 5 calibrators at different levels and including a 0.0 % calibrator for determination of the zero value. Stability of calibration: 4 weeks.

Full calibration is recommended:
- after lot change
- as required following quality control procedures

- TUC1020 HbA1c Calibration Set

SPECIFICITY / INTERFERENCES
Criterion: Recovery within ±10% of initial value.

Unconjugated and conjugated Bilirubin up to 50 mg/dL,
Ascorbic acid up to 60 mg/dL,
Lipemia (Intra-lipid) up to a triglyceride concentration of 2000 mg/dL,
RF up to 250 IU/mL,
CarbamylatedHb up to 7.5 mmol/L, and acetylated Hb up to 5.0 mmol/L.
No interference is observed by uremia, labile intermediates (Schiff base), and Haemoglobin variants HbS and HbA2. Elevated levels of HbF may lead to falsely low HbA1c values. Alcoholism and ingestion of large doses of aspirin may lead to inconsistent results.

PERFORMANCE CHARACTERISTICS
Measuring Range
The test has been developed to determine concentrations of HbA1c within a measuring range from 1.5 - 15 % according to ICC (3 – 16 % according to DCCT/NGSP), at least up to the concentration of the highest calibrator. The assay is applicable for haemoglobin concentrations in blood from 6 to 26 g/dL.

Method Comparison
A comparison between MTD Diagnostics HbA1c (y) and an immunoturbidimetric assay (x) using 109 samples gave following results (DCCT/NGSP values):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean %</th>
<th>SD %</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>3.50</td>
<td>0.05</td>
<td>1.37</td>
</tr>
<tr>
<td>Sample 2</td>
<td>8.07</td>
<td>0.11</td>
<td>1.36</td>
</tr>
<tr>
<td>Sample 3</td>
<td>11.4</td>
<td>0.21</td>
<td>1.80</td>
</tr>
</tbody>
</table>

Sensitivity / Limit of Detection: The limit of detection is 1 % HbA1c.

Precision Values (Hitachi 917) according to JDS
- Within run n=20
  - Sample 1 = 3.50 ± 0.05 (1.37)
  - Sample 2 = 8.07 ± 0.11 (1.36)
  - Sample 3 = 11.4 ± 0.21 (1.80)

- Between day n=20
  - Sample 1 = 3.65 ± 0.07 (1.82)
  - Sample 2 = 8.33 ± 0.14 (1.67)
  - Sample 3 = 11.6 ± 0.18 (1.58)

NOTES
Standardization: The assay is standardized according to the approved JDS (Japanese Diabetes Society) reference method. Calibration according to DCCT/NGSP and IFCC is also possible. Corresponding calibrator values are listed in the package insert of the calibrator set. JDS, DCCT/NGSP and IFCC values show a linear relationship and can therefore be calculated from each other using the following equation:

IFCC to JDS: JDS (%) = 0.9891 × IFCC (mmol/mol) + 1.62
IFCC to NGSP: NGSP (%) = 0.9891 × IFCC (mmol/mol) + 1.95
JDS to NGSP: NGSP = 1.02 JDS + 0.25

JCCLS: Japanese Committee Clinical Laboratory Standards
IFCC: International Federation of Clinical Chemistry
DCCT: Diabetes Control and Complications Trial
NGSP: National Glycohemoglobin Standardization Program

BIBLIOGRAPHY