Turbidimetric immunoassay for quantitative determination of HbA1c in human blood suitable for automated analyzers

**REAGENTS**

1. **Quantia HbA1c Latex Reagent (R1):** ready to use uniform suspension of latex particles.
2. **Quantia HbA1c Antibody Reagent (R2):** ready to use solution of mouse anti human HbA1c monoclonal antibody.
3. **Quantia HbA1c Antibody Reagent (R3):** ready to use solution of goat anti mouse human IgG antibody.
4. **Calibrators:**
   - **Quantia HbA1c calibrator set contains three lyophilised calibrators with different HbA1c concentrations (Level I, Level II, Level III).**
   - **Quantia HbA1c calibrator utilizes lysed human blood that is prepared from healthy non-diabetic blood donors.**
   - The elevated levels of HbA1c have been produced by controlled glycation of normal HbA1c level red cells. The % HbA1c concentration “S” for each calibrator is printed on the respective vial labels and at the end of the package insert.

The assigned % HbA1c values are lot specific and traceable to a NGSP (National Glycohaemoglobin Standardisation Programme) certified method that has documented traceability to Diabetes Control and Complications Trial (DCCT) reference method. In turn the assigned values are traceable to the International Federation of Clinical Chemists (IFCC) reference method through the master equation:

\[
\text{NGSPHbA1c} = 0.915 \times \text{IFCC HbA1c} + 2.15
\]

**ADDITIONAL MATERIAL REQUIRED**

**Specimen collection and preparation**

No special preparation of the patient is required prior to specimen collection by approved techniques. No special additives or preservatives other than anticoagulants are required. Collect venous blood in EDTA using aseptic techniques.

**Specimen Preparation**

1. Mix the specimen (sample or reconstituted calibrator or reconstituted control) thoroughly to obtain uniform distribution of erythrocytes. Avoid bubble formation.
2. Take 500 µl Quantia HbA1c Hemolysing solution in a test tube.
3. Add 10µl of homogenised specimen (sample/reconstituted calibrator/reconstituted control). Mix well and allow to stand for 15 minutes or until complete lysis is apparent. This hemolysed specimen is referred as Lyssate.

**Specimen Stability**

- Whole blood: 1 week at 2-8°C
- Lyssate: 10 hours at 15-25°C
- Lyssate: 10 days at 2-8°C

**ADDITIONAL MATERIAL REQUIRED**

Spectrophotometer with 630 nm wavelength filter and cuvette mode, well calibrated micropipettes, disposable tips, particulate free distilled water, test tube rack, incubator/waterbath set at 37°C, optically clean disposable cuvettes such as Quantiamate semi micro cuvettes/glass cuvettes, Quantia HbA1c lyophilised control (Cat. No. 108160002).

**TEST PROCEDURE**

Applications suitable for Hitachi 902, Olympus AU 400, can be made available on request.

**General Application parameter setup**

A defined application for the Quantia HbA1c Auto kit must be installed in accordance with the general instrument settings given below. For instructions refer the respective instrument manual.
Preparation of Quantia HbA1c calibration curve

1. Bring the reagents and calibrator vials to room temperature before use.
2. Reconstitute each of the calibrator vial with stated amount of hemolysing solution provided in the Quantia HbA1c kit, wait for 30 minutes, gently swirl the vial till the solution attains homogeneity.
3. The reconstituted Quantia HbA1c calibrators should be used in the same manner as specimen in accordance with reagent manufacturer’s instructions. Follow instructions accompanying the instrument and reagent kit used in the assay for specific instrument calibration procedures.
4. Increasing concentration of calibrator must be used for preparing the calibration curve.
5. Ensure that sufficient amount of calibrator lystate is prepared as per the requirement of the instrument protocol.
6. Run the calibration as per the instrument protocol.

Note: The calibration curve is usually stable for 4 weeks.

Quality control:
The calibration of Quantia HbA1c Auto Kit must be validated using Quantia HbA1c control (Cat. No.: 108160002).

Test procedure for specimen
When a valid calibration has been performed and the control is within expected range (provided in the assay value sheet) specimens can be measured. Ensure that sufficient amount of sample and reagents are present as per the requirement of the instrument protocol.

CALCULATIONS
The results are automatically calculated by the analyzer and presented as % HbA1c.

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range
The Quantia HbA1c Auto kit has been designed to measure HbA1c concentrations in the range of 4-15% in specimens. The exact range is dependent on the calibrator value which is lot specific. The Quantia HbA1c assay is linear within the measuring range.

Detection limit
The limit of detection is 3% HbA1c. The detection limit represents the lowest measurable HbA1c concentrations that can be distinguished from zero.

Precision:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Suggested applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter</td>
<td>630</td>
</tr>
<tr>
<td>Quantia HbA1c activation buffer</td>
<td>250 µl</td>
</tr>
<tr>
<td>Sample lystate</td>
<td>7.5 µl</td>
</tr>
<tr>
<td>Incubate time before addition of working reagent</td>
<td>180-300 seconds</td>
</tr>
<tr>
<td>Working reagent</td>
<td>125 µl</td>
</tr>
<tr>
<td>Read Absorbance A1 at 10-20 seconds and A2 at the end of 2 minutes</td>
<td></td>
</tr>
</tbody>
</table>

Interferences:
Bilirubin upto 60 mg/dl, Ascorbic acid upto 60 mg/dl, Triglycerides upto 1200 mg/dl, RF upto 700 IU/ml, Carbamylated Hb upto 7.5 mmol/L and Acetylated Hb upto 5 mmol/L, do not interfere in this assay.

WARRANTY
The product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY
7. Comparison of percent total GHB with percent HbA1c in people with and without known Diabetes
11. Data on file: Tulip-Diagnostics (P) Ltd.