POCD Afinion™ AS100 HbA1c METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose of this document is to describe the procedure for performing a glycated haemoglobin (HbA1c) test using the Afinion™ AS100 analyser.

2. HAZARDS

Patient Samples
All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Personal Protective Equipment should be worn when processing samples, quality control testing and maintenance procedures.

3. CLINICAL SIGNIFICANCE

Diabetes mellitus is a chronic disease characterized by high blood glucose, which results from decreased insulin production or action, or both. Type 1 diabetes mellitus (Juvenile diabetes), which accounts for 5 – 10% of diabetics, develops when the insulin-producing pancreatic beta cells are destroyed by the body’s immune system. Little or no insulin is produced, and insulin injections are required. Type 2 diabetes results when cells lose the ability to use insulin effectively (Insulin resistance). In type 2 diabetes there is also a steady decline in the number of Insulin producing beta cells, which leads to elevation of blood glucose. Control of blood glucose levels in type 2 diabetes is usually by diet, exercise, and/or oral medications.

Diabetes-related complications include diabetic retinopathy, renal failure, diabetic neuropathy, foot ulceration, lower-limb amputation and heart disease. Commonly tested markers for monitoring diabetes and its side effects are blood glucose, HbA1c and urinary albumin. The blood glucose test measures the level of glucose in the patient’s blood at the time the sample is taken. The HbA1c is an indicator of long-term glycaemic control and urinary albumin monitors renal damage caused by the diabetes.

HbA1c is formed by non-enzymatic glycation of the N-terminal of the B-chain of haemoglobin in red blood cells. During the normal 120-day lifespan of a red blood cell, glucose molecules react with the haemoglobin, forming HbA1c. The amount of HbA1c formed is directly related to the average blood glucose level. HbA1c is stable – once a haemoglobin molecule is glycated, it remains so, and therefore HbA1c can indicate glycaemic control over a 2-3 month period. In non-diabetic subjects, 4-6% of haemoglobin is glycated, while uncontrolled diabetics may exhibit levels in excess of 15%.

In 1993, the Diabetes Control and Complications Trial (DCCT) established that the development and progression of complications of type 1 diabetes was closely related to A1c levels. Similarly, the United Kingdom Prospective Diabetes Study demonstrated that lowering blood glucose levels in type 2
4. TEST PRINCIPLE

HbA$_{1c}$ point of care devices generally use one of the following methods: immunoassay, boronate affinity chromatography or micro optical detection. In the US the tests are certified by the National Glycohaemoglobin Standardisation Program (NGSP) to standardize them against the results of the 1993 DCCT.

The Afinion™ AS100 analyser uses boronate affinity technology. The blood sample is automatically mixed with a fluid that releases haemoglobin from the erythrocytes. The haemoglobin precipitates. The sample mixture is transferred to a blue boronic acid conjugate which binds to the cis-diols of HbA$_{1c}$. The reaction mixture is soaked through a filter membrane and all precipitated haemoglobin, conjugate-bound and unbound (glycated and non-glycated) remains on the membrane. Excess conjugate is removed with a washing reagent.

The Afinion™ AS100 analyser evaluates the precipitate on the membrane. By measuring the reflectance of the blue (HbA$_{1c}$) and red (total haemoglobin) colour intensities, the ratio between them being proportional to the percentage of HbA$_{1c}$ in the sample. The HbA$_{1c}$ concentration is displayed on the Afinion™ analyser in the units mmol/mol, percentage (%), as the calculated estimated average glucose (eAG) or combinations of these.

4.1 Analytical Specificity
The AfinionTM HbA1c test measures the total glycated haemoglobin and reports the HbA1c value. The following Hb variants have been analysed and found not to affect the AfinionTM HbA1c test result. HbAC, HbAD, HbAE, HbF, HbAJ and HbAS. Carbamylated and pre-glycated haemoglobin does not affect the AfinionTM HbA1C test result.

4.2 Interference
No significant interference was observed by the following at the concentrations indicated:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>342 umol/L</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>15.7 mmol/L</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>9.1 mmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>27.8 mmol/L</td>
</tr>
<tr>
<td>Fructosamine</td>
<td>680 umol/L</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>3.3 mmol/L</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.8 mmol/L</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>4.3 mmol/L</td>
</tr>
<tr>
<td>Glyburide</td>
<td>3.9 umol/L</td>
</tr>
<tr>
<td>Metformin</td>
<td>310 umol/L</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>1.7mmol/L</td>
</tr>
<tr>
<td>Haemolysis (in vitro)</td>
<td>5%</td>
</tr>
</tbody>
</table>

Important! It is possible that other substances and/or factors not listed above may interfere with the test and cause false results.

4.3 Limitations of the test
Diluted samples cannot be used with AfinionTM HbA1c. Coagulated or haemolysed samples cannot be used with AfinionTM Hba1c. If the sample has a haemoglobin result below 60 g/L or above 200 g/L, no test result will be reported and an information code will be displayed.
In patients with severe anaemia or conditions that alter the red cell life span HbA1c will not reflect glycaemic control.

4.4 Accuracy

This product fulfills the requirements of the EU Directive 98/79/EC on in vitro diagnostic medical devices. A method comparison study, comprising 39 blood samples (4.9-11.7% HbA1c) were analysed with an affinity HPLC system and Afinion™ AS100 Analyser. A linear regression analysis and a Bland-Altman analysis were performed.

<table>
<thead>
<tr>
<th>N</th>
<th>Regression line</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>y = 0.96x + 0.33</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N</th>
<th>Bias</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>0.00% HbA1c</td>
<td>-0.31-0.30% HbA1c</td>
</tr>
</tbody>
</table>

4.5 Precision

Within-run, between-day and total precision values were determined according to CLSI Protocol EP5-A. Afinion™ HbA1c, Control C I, Control C II, one EDTA sample assayed for 20 days and one EDTA sample assayed for 17 days. The samples were analysed in duplicate twice a day.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean % HbA1c</th>
<th>Within-run CV (%)</th>
<th>Between-day CV (%)</th>
<th>Total CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control C I</td>
<td>20</td>
<td>6.5</td>
<td>0.9</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Control C II</td>
<td>20</td>
<td>9.1</td>
<td>0.6</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Patient 1</td>
<td>17*</td>
<td>5.6</td>
<td>0.9</td>
<td>0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Patient 2</td>
<td>20</td>
<td>10.0</td>
<td>0.7</td>
<td>0.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Based on 17 days of analysis due to hemolysis of the sample.

5. INSTRUMENT
## Product Specifications

### 5.1 Operating Conditions and Technical Data

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range</td>
<td>18° – 30° C</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>10-90% non condensing</td>
</tr>
<tr>
<td>Maximum altitude</td>
<td>Not specified</td>
</tr>
<tr>
<td>Position</td>
<td>Place the meter on a clean, dry, level, vibration-free surface. The device must be placed to allow maximum airflow surrounding the device to prevent over heating. Avoid direct sunlight.</td>
</tr>
</tbody>
</table>
| Measuring range                        | HbA$_{1c}$ (%): 4.0 – 15.0  
HbA$_{1c}$ (mmol/mol): 20 - 140  
eAG (mmol/L): 3.8 - 21.3 |
| Sample size                            | 1.5µl in the glass capillary of the sampling device                     |
| Test time                              | 3 minutes                                                               |
| Memory                                 | 500 patient results and 500 control results with date and time          |
| Barcode scanner                        | Yes                                                                     |
| Interface                              | 2x USB ports, serial port                                               |
| Battery operation                      | No                                                                      |
| Mains connection                       | Separate AC to DC mains adapter. Double insulated                       |
| Number of tests per set of batteries   | NA                                                                     |
| Safety class                           | Not stated                                                              |
| Automatic power-off                    | Yes. Device can remain on at all times.                                  |
| Dimensions                             | 320 x 170 x 170 mm                                                      |
| Weight                                 | 5kg                                                                     |

### 5.2 Storage and transport conditions

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range</td>
<td>-40° - 70° C</td>
</tr>
<tr>
<td>Meter (In original container)</td>
<td></td>
</tr>
<tr>
<td>Relative humidity</td>
<td>10 - 93% at 40°C</td>
</tr>
</tbody>
</table>
6. SPECIMEN REQUIREMENTS

6.1 Sample Material
Fresh capillary whole blood or venous whole blood collected in EDTA, heparin, sodium citrate, or sodium fluoride tubes are acceptable.

Capillary blood (from finger prick)
- Wash hand with warm soapy water and dry thoroughly prior to collecting samples.
- Before performing capillary puncture, stimulate blood flow in the fingertip by warming the finger.
- Puncture a non-callused area of the fingertip of the middle or ring finger
- Use a lancing device that provides a deep puncture so that blood flows freely.
- Immediately after lancing, massage gently along the side of your finger to obtain a drop without pressing or squeezing too hard.

Venepuncture (see suitable anticoagulants above)
- Skin surface must be cleaned with an alcohol swab and dried well prior to collection to ensure there are no substances on the skin surface
- Mix the sample well by inverting the tube 8 – 10 times before sampling.
- Venous whole blood with anticoagulants may be stored refrigerated at 2 - 8°C for 10 days.
- If refrigerated, patient samples should be allowed to equilibrate to room temperature.

7. CARTRIDGES/REAGENTS

1a Sampling device - closed position
1b Sampling device – lifted position
2 Capillary
3 Reaction Wells
   a) Conjugate
   b) Membrane tube
   c) Washing Solution
   d) Buffer Solution
   e) Empty
4 Handle
5 Barcode Label
6 Optical reading area
7 ID Area
All reagents required for one test are present in the test cartridge: Boronic acid conjugate, Polyethersulfone membrane, Morpholine buffered sodium chloride washing solution with detergents and preservative, HEPES buffered sodium chloride reconstitution reagent with lysis and precipitation reagents.

The test cartridge and the sampling device have a color unique to the test being performed. The test cartridges are separately packed in foil pouches to protect the chemicals and plastic devices from light, dirt and humidity. The reagents are all ready for use. An integrated sampling device is used for the collection of the sample or control. The test cartridge cannot be re-used.

7.1 Storage and handling
• Test cartridges are stable until the expiry date only when stored refrigerated (2 - 8°C) in individually sealed foil pouches (do not freeze). The expiry date is the last day of the month stated on the foil pouch and kit container.
• Test cartridges can be stored in unopened foil pouches at room temperature (15 - 25°C) for 90 days cumulative (i.e. 2 months at room temperature, then stored in the fridge and back at room temperature for 1 month). Note the date of removal from the refrigerator on the kit container.
• Do not store test cartridges close to heat sources or exposure to direct sunlight
• Open the foil pouch just before use as the test cartridge is sensitive to humidity: at relative humidity ≤ 60% the Test Cartridge must be used within 30 minutes; at relative humidity > 60% the Test Cartridge must be used within 10 minutes.
• Avoid relative humidity above 90% (non-condensing).
• Ensure cartridges and controls are at room temperature before use

8. CALIBRATION
The Afinion™ HbA₁c is traceable to the IFCC reference method for HbA₁c. During manufacture, Afinion™ AS100 analysers are calibrated against a reference system. This procedure has been established for each lot of test cartridges and then stored in the barcode label. When the cartridge enters the analyser, the integrated camera reads the barcode. The calibration data for the actual lot are transferred to the instrument and used for calculating the results. Calibration by the operator is thus not required. Each device is set to perform a 3 minute self-calibration check daily.

9. QUALITY CONTROL

9.1 Quality control material (perform as per your organisation protocol)
Accurately testing known levels of HbA₁c ensures that the system and your technique used in testing give accurate results on patient tests. The control solutions have defined (known) values. The results for these solutions must first fall within a certain acceptable range in order to allow valid patient testing.

A quality control test should be performed every time a new shipment of test cartridges are received, when a new lot number of test cartridges are used, if the
clinical picture does not correlate with the patient test results, after major maintenance, and at a minimum of once a month.

Enrolling in an External Quality Assurance Program is encouraged to objectively compare results with other users using the same method of testing. If an External Quality Assurance Program is not available, monthly lab comparison is encouraged.

### 9.2 Laboratory Comparison

If your policy states you must perform laboratory comparison then perform a venepuncture sample for the laboratory and store in an EDTA, heparin, sodium citrate, or sodium fluoride tube. Perform a capillary collection and run a sample on the POCD Afinion AS100. Collection of both the venepuncture and capillary sample should occur at the same time. Record and compare the results ensuring they are in acceptable range for your organisation.

### 9.3 Running a control solution

The control solutions has two level:
- Axis-Shield Afinion HbA1c Control, level 1 - low
- Axis-Shield Afinion HbA1c Control, level 2 - high

Store the controls at 2 - 8°C. The expiry date of the kit only applies for unopened product stored refrigerated (2-8°C). The expiry date is the last day of the month stated on the outer container. Avoid exposure to direct sunlight and temperatures above 25°C. Do not freeze. Opened control vials are stable for 60 days when stored refrigerated (2-8°C). It is recommended to note the date of opening on the vial label. Always store the control vials refrigerated in an upright position refrigerated (2-8°C) when not in use.

#### Preparing the control solution

- Allow the control material to reach room temperature (15-25°C) before use, which takes approximately 45 minutes.
- Allow the unopened test cartridge to sit at room temperature for at least 15 minutes prior to testing.
- Mix the control material thoroughly by swirling the vial sideways for 30 seconds until all the sediment is dissolved from the base of the vial. This is very important.

#### Applying control solution to the sampling device

- Open the foil pouch just before use. Use the handle for correct grip. Avoid touching the optical reading area of the cartridge.
- Inspect the cartridge. Do not use the cartridge if it is damaged or covered with loose desiccant particles.
- With the test cartridge sitting on table, hold the cartridge by the handle and with the other hand; pull the sampling device straight up from the test cartridge.
- Invert the control solution another 6 times and extract the sample from the cap with the capillary device drawing the sample from just below the surface of the sample. Do not wipe off the capillary. Avoid air bubbles or excess sample on the outside of the capillary.
• Immediately replace the sampling device carefully into the test cartridge.
• NOTE: The test cartridge must be analysed within 1 minute from capillary filling.

**Inserting test cartridge into the analyser**
• To analyse the control, touch the control button to enter control mode.
• The lid will open automatically.
• Insert the test cartridge with the barcode facing left.
• A “C” in the upper left corner of the display indicates that the analyser is in control mode.
• Close the lid manually. The analyser will start processing the cartridge.
• Touch the control ID button and enter the control ID.
• Touch the Return button to confirm.
• NOTE: Control ID MUST always be added prior to cartridge being ejected.

**Results**
• Record the result and touch the Tick button to accept.
• If a printer is connected, touch the print button to print the result.
• Check that the control result is within the acceptable range.
• If the control result is not acceptable, patient testing must not be performed until corrective action has been undertaken and control results are back in range.

### 10. TEST PROCEDURE

If you are required to disconnect the device from the power, always use the power button to turn off. Do not unplug the power to turn the device off.

**Preparing the analyser**
• Allow the unopened test cartridge to sit at room temperature for at least 15 minutes prior to testing.
• Be sure that the analyser is switched on and ready for use.
Applying blood to the sampling device

• Open the foil pouch just before use. Use the handle for correct grip. Avoid touching the optical reading area of the cartridge.
• Inspect the cartridge. Do not use the cartridge if it is damaged or covered with loose desiccant particles.
• With the test cartridge sitting on table, hold the cartridge by the handle and with the other hand; pull the sampling device straight up from the test cartridge.

![Image of blood collection]

• Fill the capillary completely with patient sample by holding the sampling device at an acute angle and bringing the capillary tip just beneath the surface of the sample. Do not wipe off the capillary. Avoid air bubbles or excess sample on the outside of the capillary.

![Image of capillary filling]

• Immediately replace the sampling device carefully into the test cartridge. **NOTE:** The test cartridge must be analysed within 1 minute from capillary filling.

Inserting test cartridge into the analyser

• Touch the top button to begin a patient test. The lid opens automatically. **NOTE:** Never attempt to manually open the lid.
• Insert the test cartridge with the barcode label facing left and close the lid manually.
  **NOTE:** The cartridge will only fit in one way

• Touch the patient ID button and enter patient ID during processing (if activated). Touch RETURN to confirm.
  **NOTE:** Patient ID MUST be added prior to cartridge being ejected.

**Results**

• A status bar will appear while the sample is being processed
• The result will appear on the screen.
• Record the result, then touch TICK to accept. The lid opens automatically.
• Remove and discard the used test cartridge. Close the lid manually.
  **NOTE:** Once the test has been performed, the used test cartridge is shorter in size than an unused cartridge.

11. **RESULTS**

The normal range for HbA\textsubscript{1c} in non-diabetic people is 4 to 6\% (DCCT).\textsuperscript{7} The American Diabetes Association recommends a goal of <7\% for effective management of diabetes and to minimize long-term complications.\textsuperscript{1}
A level above 7\% suggests that more intensive diabetes management should be considered.\textsuperscript{8}

12. **MAINTENANCE**

No maintenance of the Afinion\textsuperscript{TM} AS100 analyser is required other than cleaning the exterior and cartridge chamber. Do not use any cleaning liquid or equipment other than those recommended. Do not immerse the Analyser in water or other liquids. If a software update is needed, this will be done via USB.

**Cleaning the exterior**

• Cleaning the exterior of the Afinion\textsuperscript{TM} AS100 Analyser should be performed whenever necessary.
• Most spills and stains can be removed with water or a mild detergent.
• Switch off the Analyser using the power button. Unplug the power supply when the shut down procedure is completed.
• Clean the outside of the Analyser and the touch display with a clean, lint-free and non-abrasive cloth dampened in water or a mild detergent.
• To disinfect the exterior of the instrument, first remove as much as possible of the spilled material with a cloth dampened in the disinfectant (2% glutaraldehyde or 0.5% sodium hypochlorite).
• The surface of the Analyser should be exposed to the disinfectant for at least 10 minutes.
• Allow the Analyser to air dry.
• Plug in the power supply and switch on the Analyser.

Cleaning the cartridge chamber
• The Afinion™ AS100 Analyser Cleaning Kit should always be used for cleaning the cartridge chamber.
• The cartridge chamber should be cleaned immediately if materials or liquids are spilled in the cartridge chamber. For regular maintenance (removal of dust particles etc.), the cartridge chamber should be cleaned every 30 days.
• Touch to open the lid.
• Unplug the power supply.
• Wet a Cleaning Swab with 3 drops of water or a disinfectant (2% glutaraldehyde or 0.5% sodium hypochlorite). Do not soak.
• Carefully remove spills and particles from the cartridge chamber using the moistened swab.
• To disinfect the cartridge chamber, the surface of the chamber should be exposed to the disinfectant for at least 10 minutes.
• Wipe off any residual liquid from the cartridge chamber using a new, dry Cleaning Swab.
• Close the lid.
• Plug in the power supply and switch on the Analyser.
• Do not allow liquid to drip off the Cleaning Swab into the Analyser. If liquid drips into the Analyser, optics can be destroyed.

13. REFERENCES

This method has been adapted from the Afinion™ AS100 Analyser user manual and the Afinion™ HbA1c test cartridge and control solution package inserts.

monitoring>.