AXIS-SHIELD HbA1c Reagent Kit

REF FHHBA100 | Σ Reag 1, 1 x 40.8 mL; Reag 2, 1 x 17.1 mL; Lysis Diluent, 4 x 60.0 mL | IVD | CE

ENGLISH

FOR PROFESSIONAL USE ONLY

INTENDED USE

The HbA1c assay is an Immunoturbidimetric immunoassay for the quantitative determination of percent haemoglobin A1c (HbA1c) in human whole blood on clinical chemistry analysers. Percent HbA1c measurements are used for monitoring long-term glycaemic control in diabetic patients.

SUMMARY

HbA1c is formed by the reaction of glucose with the N-terminal amino group of the haemoglobin beta chain. The Diabetes Control and Complications Trial (DCCT) Research Group previously reported a relationship between percent HbA1c and mean blood glucose levels during the preceding 2-3 months[1]. The DCCT study also demonstrated that long-term control of diabetes can prevent complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy.
Measurement of percent HbA1c is the method of choice for monitoring therapy of diabetic patients[2,3].

PRINCIPLE

The Axis-Shield HbA1c assay utilises the interaction of antigen and antibody to directly determine the concentration of HbA1c (%) in whole blood. Whole blood samples are treated with Lysis Diluent to lyse the red blood cells. Lysed sample is then incubated with latex microparticles (Reag 1). Haemoglobin and HbA1c are captured on the microparticles. When anti-HbA1c (mouse monoclonal) antibody (Reag 2) is added, latex-HbA1c-antibody complex is formed. The amount of agglutination is measured turbidimetrically and is proportional to the amount of HbA1c absorbed on the surface of the microparticles.

KIT COMPONENTS

REAG 1	1 x 40.8 mL	Latex microparticles 0.13%, buffer, stabilisers. **READY TO USE**	
REAG 2	1 x 17.1 mL	Mouse anti-human HbA1c (monoclonal) 0.04mg/mL, anti-mouse (polyclonal) IgG 0.06 mg/mL, buffer, stabilisers (Proclin 300) **READY TO USE** **NB. WARNING**	
LYSIS DILUENT	4 x 60.0 mL	Buffer, stabilizers (sodium azide, <0.01%). **READY TO USE**	

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- General laboratory equipment
- Distilled or RO water
- Axis-Shield HbA1c Calibrator Kit, Product Code FHHBA300
- Axis-Shield HbA1c Control Kit, Product Code FHHBA200

WARNINGS AND PRECAUTIONS

1. IVD For in vitro diagnostic use.
2. Disposal of all waste material must be in accordance with local guidelines.
3. Lysis Diluent contains sodium azide which can react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large quantities of water to prevent azide build-up.
4. Material safety data sheets are available upon request from Axis-Shield.

 REAG 2 WARNING	**WARNING** H315 – H317 – H319 – **PREVENTION** P264 – P280 –	Causes skin irritation. May cause an allergic skin reaction. Causes serious eye irritation. Wash hands thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection.

 REAG 2 WARNING	**RESPONSE** P305+351+338 – P332+313 – P362 –	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse.

REAGENT STORAGE AND STABILITY

Opened (in-Use) Kit Stability

- Lysis Diluent:

Stable for 60 days at 2-8°C after initial opening. Product must not be stored at room temperature for longer than 30 hours during use.

- Reag 1 and Reag 2:

Stable when stored on-board a refrigerated analyser for up to 28 days, if contamination is avoided. If reagents are removed from the analyser return to storage at 2-8°C. After 28 days, the reagents must be discarded.

Unopened kit stability

All components are stable until expiration as directed on the label at 2-8°C. Reagents must not be frozen.

Handling and Procedural Notes

- Store kit components at 2-8°C.
- Reagents are supplied ready for use.
- Do not use beyond the expiration date.
- **DO NOT FREEZE REAGENTS.**
- Reagents must be returned to 2-8°C storage after use if they cannot be stored on-board.
- Do not mix reagents between different lot numbers.
- Use a new disposable pipette tip for each reagent or sample manipulation to avoid contamination of reagents.
- Reagents should be clear of particulate material and should be discarded if they become turbid.

Indications of Deterioration

Turbidity or precipitation in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the Axis-Shield HbA1c controls may also be an indication of reagent instability and associated results are invalid. Samples should be retested.

SAMPLE STORAGE, COLLECTION AND HANDLING

Collection and Handling

- For sample collection and preparation, only use suitable tubes or collection containers.
- Only the samples listed were tested and found suitable for use:
  - Dipotassium EDTA (K2-EDTA)
  - Lithium Heparin (Li-Heparin)
- Other sample collection tubes have not been verified for use.
- Do not use samples with the following conditions: heat-inactivated, pooled, obvious microbial contamination.
- Caution when handling patient samples in order to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each sample to prevent cross contamination.

Preparation for analysis

- When processing samples follow the instructions provided by the collection tube manufacturer.
- All human samples should be considered potentially infectious. It is recommended that these materials be handled in accordance with local/national guidelines on laboratory safety procedures.
- Fresh/Non-Frozen Samples:
  - Do not centrifuge fresh/non-frozen samples.

- Samples must be mixed thoroughly prior to use.
- Frozen Samples:
  - Allow samples to thaw for a minimum of 30 minutes.
  - Mix thawed samples thoroughly by inverting 4 times.
  - Visually inspect the samples. If layering or stratification is observed, continue mixing until they are visibly homogeneous.
  - To ensure consistency in results, frozen and thawed samples must be transferred to a centrifuge tube and centrifuged at ≥10,000 RCF (Relative Centrifugal Force) for 5 minutes before testing.

Manual Haemolysate Preparation

To determine the level of HbA1c, a haemolysate must be prepared for each sample:

- Dispense 1mL of Lysis Diluent into appropriately labelled tubes.
  Note: Plastic or glass tubes of appropriate size are acceptable.
- Place 10 µL of mixed whole blood sample into the appropriately labelled lysis reagent tube.
- Mix thoroughly by gentle vortexing for 30 seconds.
- Allow samples to stand for 2 minutes until complete lysis is evident.
- Immediately after use close the Lysis Diluent bottle.

Sample Storage and Stability

- Whole Blood samples are stable when stored up to:
  - 6 hours at room temperature
  - 5 days at 2-8°C or 14 days at -20°C
  - Avoid more than one freeze/thaw cycle.
- Lysate (Haemolysate) are stable when stored up to:
  - 6 hours at room temperature
  - 5 days at 2-8°C
  - 3 weeks at -20°C
  - Avoid more than one freeze/thaw cycle.
  - When stored on-board the instrument testing must not be delayed more than 2 hours.

ASSAY PROCEDURE

- Programme the instrument using the appropriate instrument-specific protocol. Refer to Analytical Procedure section.
- Load reagents and samples as per instrument requirements.

STANDARDIZATION

The Axis-Shield HbA1c assay is traceable to the International Federation of Clinical Chemistry (IFCC) reference standards.

CALIBRATION

For assay calibration use the Axis-Shield Calibrator materials as listed in the "Materials required" section.
The Calibrator values are lot-specific as directed on the labels.

Calibration Frequency:

Calibration is stable for up to 14 days.
Recalibration is also required after a change in reagent lot, if a control reads out-of-range or as required following quality control procedures.

QUALITY CONTROL

For quality control, use Axis-Shield Control materials as listed in the "Materials required" section. Maintenance and calibration of the instrument must be performed according to the operator's instruction manual for the specific analyser.
Users should ensure they understand the instructions of this assay, particularly the Warnings and Precautions, Sample Handling and Procedure Limitations sections. It is recommended that Axis-Shield HbA1c Controls and Calibrators are run in duplicate each day of use.
The control limits should be established by individual laboratories and in accordance with laboratories quality control procedures and/or any local or government guidelines.

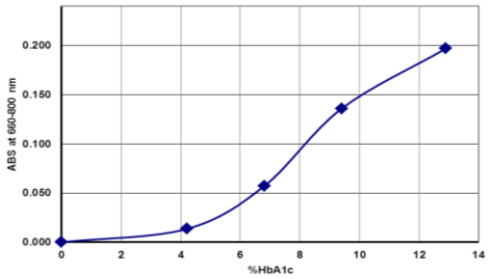
RESULTS

Unit of Measure

The default result unit for the assay is % HbA1c.
For alternative units, manual calculations can be used according to the following equations:

- NGSP %HbA1c to IFCC mmol/mol: [%HbA1c x 10.93] - 23.50
- IFCC mmol/mol to NGSP %HbA1c: [mmol/mol x 0.09148] + 2.152

HbA1c results are calculated from a spline data reduction method to generate a calibration curve. An example curve is illustrated below:



MEASURING RANGE

The analytical measuring range of the assay is the range from the functional sensitivity of the assay to the concentration (%HbA1c) of the highest Calibrator. The range of the Axis-Shield HbA1c assay is 4.0% to 13.0 % (*) HbA1c.
(*) Values vary depending on the specific calibrator lot target values and this must be taken into consideration during the instrument settings for the assay range limits. The range stated has been based on typical high calibrator value.
Samples above the measuring range **MUST not be diluted**. These samples should be tested with alternative methods.

EXPECTED VALUES

For monitoring diabetic patients, it is recommended that glycaemic goals are individualized following current professional society recommendations[4+]. The American Diabetes Association (ADA) recommendations are summarized in the following table.

HbA1c Value	Glycaemic Goal
< 8% HbA1c (64 mmol/mol)	Less Stringent
< 7% HbA1c (53 mmol/mol)	General (Non-Pregnant Adults)
< 6.5% HbA1c (48 mmol/mol)	More stringent

As recommended by the ADA, patients in the range of 5.7 - 6.4 %HbA1c (39 - 46 mmol/mol) would be in the category of increased risk for diabetes[4].

PROCEDURE LIMITATIONS

- Haemoglobinopathies may interfere with glycated haemoglobin analysis. Common haemoglobin variants have been tested in this assay. Refer to the Specificity section.
- It is possible that other substances and/or factors not tested (refer to Interferences section) may interfere with the assay.
- This assay is not intended for:
  - The diagnosis of diabetes.
  - should not be used to replace daily home testing of urine and blood glucose levels.
  - Analysing samples from patients with total haemoglobin levels < 8g/dL as results will be negatively biased.
  - analysing samples from patients with conditions causing shortened red cell survival time such as haemolytic anemia, or other haemolytic disease, significant acute or chronic blood loss or pregnancy.

PERFORMANCE CHARACTERISTICS:

Representative data in this section was generated from testing on the ADVIA 2400 analyser; results from individual laboratories may vary.
The performance of other instrument applications have not been verified so must be verified by the user.

FUNCTIONAL SENSITIVITY

A study was performed to assess the performance of the assay at low HbA1c levels. Three low level whole blood samples were tested (n=20) on 3 testing occasions using 3 Axis-Shield HbA1c Reagent lots. Sample concentrations ranged from 2.9 to 4.6 %HbA1c. All sample SD were < 0.24 %HbA1c.
The functional sensitivity of the Axis-Shield HbA1c assay is 4.0 %HbA1c.

ASSAY PRECISION

Total assay imprecision was performed in an external study using controls and human whole blood samples. All samples were assayed in duplicate (n=2) at 2 separate times per day for 20 days using 3 Axis-Shield HbA1c Reagent lots. Minimum observed performance data for samples using one reagent lot are summarized in the following table.

			Total Imprecision	
Sample	**n**	**Mean (%)**	**SD (%HbA1c) (95% CI)**	**CV% (95% CI)**
Control Low	80	6.17	0.218 (0.177 – 0.283)	3.5 (2.9% - 4.6%)
Control High	80	9.97	0.587 (0.484 – 0.740)	5.9 (4.9% – 7.5%)
Human Blood #1	80	6.32	0.254 (0.207 – 0.329)	4.0 (3.3% - 5.2%)
Human Blood #2	80	7.60	0.317 (0.273 – 0.378)	4.2 (3.6% - 5.0%)
Human Blood #3	80	8.57	0.366 (0.313 – 0.441)	4.3 (3.6% - 5.1%)

LINEARITY

A linearity study was performed with serial dilutions of a high %HbA1c sample with a low %HbA1c sample. Testing was performed using 3 Axis-Shield HbA1c Reagent lots.

The Axis-Shield HbA1c assay demonstrated linearity from 5.5 - 11.0 %HbA1c.

METHOD COMPARISON

A correlation study was performed using human whole blood samples across the assay range. Samples were analysed using 3 Axis-Shield HbA1c reagent lots and two commercially available HbA1c assays (an immunoassay and a Capillary Electrophoresis Method). The samples were evaluated using the Passing-Bablok regression method and the correlation was assessed by Pearson (r) regression method. The following results were obtained:

- Immunoassay Method Comparison

Lot	**Sample Range (%HbA1c)**	**n**	**Correlation Coefficient (r)**	**Slope (95% CI)**	**Intercept (95% CI)**
1	4.2% - 12.6%	154	0.968	0.99 (0.96 – 1.02)	0.76 (0.58 – 0.95)
2	4.2% - 12.6%	154	0.968	0.94 (0.91 – 0.99)	1.00 (0.72 – 1.27)
3	4.2% - 12.6%	154	0.976	0.98 (0.95 – 1.02)	0.77 (0.52 – 0.99)

- Capillary Electrophoresis Method Comparison

Lot	**Sample Range (%HbA1c)**	**n**	**Correlation Coefficient (r)**	**Slope (95% CI)**	**Intercept (95% CI)**
1	4.4% - 13.0%	157	0.973	0.95 (0.92 – 0.98)	0.45 (0.25 – 0.66)
2	4.4% - 13.0%	158	0.964	0.92 (0.88 – 0.96)	0.59 (0.28 – 0.91)
3	4.4% - 13.0%	158	0.976	0.94 (0.90 – 0.98)	0.54 (0.27 – 0.80)

INTERFERENCES

The compounds listed below were spiked into two human whole blood samples with different levels of %HbA1c (6-7% and 8-9%HbA1c) to assess for potential interference in the assay when compared to reference samples.

None of the substances at the levels indicated showed interference in the Axis-Shield HbA1c assay as defined by a < ±10% deviation in concentration:

Potential Interferent	**Highest test level with no interference**
Bilirubin (Conjugated & Unconjugated)	38 mg/dL
Triglycerides	1000 mg/dL
Rheumatoid Factor	600 U/mL
Total Proteins	14 g/dL
Ascorbic Acid	50 mg/dL

Care should be taken when testing samples from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy as they may contain anti-mouse antibodies (HAMA). Samples containing HAMA may give unexpected values when tested in this assay as with any assay employing mouse antibodies.[5]

+SPECIFICITY

Haemoglobin (Hb) derivatives

Labile fractions Hb, Acetylated Hb and Carbamylated Hb do not interfere in this assay.

Two human whole blood samples with %HbA1c concentrations of 6-7% and 8-9%HbA1c were tested in the presence of physiological or recommended test concentrations of Sodium Cyanate, Acetylsalicylate, Glucose and Urea and no bias as defined by a maximum deviation in concentration of < ±10% compared to reference samples was reported.

Haemoglobin variants

The following variants do not interfere in the assay: HbS, HbC, HbD, HbA2, HbE and HbF. Other variants have not been tested in the assay.

As a matter of principal, care must be taken when interpreting any HbA1c result from patients with elevated levels of Hb variants.

BIBLIOGRAPHY

1. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Eng J Med* 1993;329(14):977-86
2. Lester E. The clinical value of glycated haemoglobin and glycated plasma proteins. *Ann Clin Biochem* 1989;26: 213-9.
3. Goldstein DE, Little RR, Weidmeyer H-M, *et al*. Glycated hemoglobin: methodologies and clinical applications. *Clin Chem* 1986; 32(10):B64-B70.
4. American Diabetes Association. Position Statement: Standards of Medical Care in Diabetes - 2012. In: Diabetes Care 2012;35 (Suppl 1):S11–S63.
5. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. Clin Chem 1988;34(2):261-264.

TYPICAL ANALYTICAL PROCEDURE

Lysed whole blood volume:	4 µL
Reagent 1 volume:	150 µL
Incubation time:	5 minutes
Reagent 2 Volume:	60 µL
Incubation time:	5 minutes
1st Reading time:	20 seconds (15 -25 seconds)
2nd Reading time:	300 seconds (270-330 seconds)
Wavelengths:	660 nm (640-680 nm)
Temperature:	+ 37 C
Type of reaction:	End point
Type of calculation:	Spline
Calibration Model:	Multipoint

Refer to the instrument-specific operating manual for instrument programming instructions

REF	Product Code
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
Σ	Number of tests
	Consult Instructions For Use
	Manufacturer
GTIN	Global Trade Item Number
	Use by
8°C 2°C	Store at 2-8°C
	CAUTION
REAG 1	Reagent 1
REAG 2	Reagent 2
LYSIS DILUENT	Lysis Diluent

Axis-Shield Diagnostics Ltd.,
The Technology Park,
Dundee, DD2 1XA
United Kingdom
Tel: +44 (0) 1382 422000
Fax: +44 (0) 1382 422088

RPBL1087/R3
Ver 2015/04