

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k072686

B. Purpose for Submission:

New Device

C. Measurand:

Hemoglobin A1c (HbA1c) assay, calibrators, and controls

D. Type of Test:

Quantitative Immunoassay

E. Applicant:

Axis-Shield Diagnostics, Ltd.

F. Proprietary and Established Names:

AxSYM HbA1c

G. Regulatory Information:

1. Regulation section:

21 CFR 864.7470 – Glycosylated hemoglobin assay

21 CFR 862.1150 – Calibrator

21 CFR 862.1660 - Quality Control Material (Assayed and Unassayed).

2. Classification:

Class II, Class II

3. Product code:

LCP – Glycosylated hemoglobin assay

JIT – Secondary calibrator

JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

Hematology (81) and Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

Reagents

The AxSYM HbA1c assay is an immunoassay for the quantitative determination of percent hemoglobin A1c (HbA1c) in whole blood samples on the AxSYM System. Percent HbA1c measurements are used in the clinical management of diabetes to assess the long-term efficacy of diabetic control.

Calibrators

The AxSYM HbA1c Standard Calibrators are for the standard calibration of the AxSYM System when used for the quantitative determination of percent HbA1c in whole blood samples.

Controls

The AxSYM HbA1c Controls are for the use in quality control to monitor the accuracy and precision of the AxSYM HbA1c assay when used for the quantitative determination of percent hemoglobin A1c (HbA1c) in whole blood samples on the AxSYM System.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Abbott AxSYM

I. Device Description:

The AxSYM HbA1c Reagents, Standard Calibrators and Controls are designed to be used in the AxSYM HbA1c assay on the AxSYM system. The AxSYM HbA1c assay is an immunoassay for the quantitative determination of percent hemoglobin A1c (HbA1c) in whole blood samples on the AxSYM System.

Each AxSYM HbA1c Reagent kit contains 1 bottle of each component of Conjugate, Sample Diluent, Blocking Buffer and Lysis Buffer

- Conjugate: Anti-HbA1c Antibody: Alkaline Phosphatase Conjugate in TRIS buffer with protein and preservative.
- Sample Diluent in TRIS buffer with preservative.
- Blocking Buffer in TRIS buffer with preservative.
- Lysis Buffer containing detergent and preservative.

Each AxSYM HbA1c Standard Calibrator kit contains six Bottles (2.0 mL each) of AxSYM HbA1c Standard Calibrators. Calibrators A-F contain processed human blood with a preservative. Each AxSYM HbA1c Control kit contains two bottles (5.0 mL each) of AxSYM HbA1c Controls. Controls L and H contain processed human blood and a preservative. The human whole blood used in the calibrators and controls is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, Anti- HIV-1/HIV-2, and anti-HCV or HCV RNA.

J. Substantial Equivalence Information:

1. Predicate device name(s):

G7 Automated Glycosylated Hemoglobin HPLC Analyzer

2. Predicate K number(s):

k011434

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Specimen Type	Human whole blood	Human whole blood
Calibration	Calibrators are traceable to the IFCC reference calibrators	Calibrators are traceable to the IFCC reference calibrators

Differences		
Item	Device	Predicate
Technology	Immunoassay	HPLC
Reagent Storage	2-8°C	4-15°C
Calibrator and Control Storage	-20°C or colder	2-8°C
Calibrator and Control in-use storage conditions	Calibrator Pack and Control Pack can be stored at 2-8 °C for up to 30 days	Calibrator and Controls can be stored at 2-8 °C for up to 7 days

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

In the AxSYM HbA1c assay, a whole blood sample is lysed, releasing hemoglobin and HbA1c. Hemoglobin and HbA1c analyte are captured on a glass fiber matrix by a binding reaction that occurs between the analyte and a Blocking Buffer. HbA1c is quantified by measuring the amount of HbA1c analyte captured on the matrix cell, using a conjugate of Anti-HbA1c and Alkaline Phosphatase as the signal-generating molecule, and the substrate, 4-Methylumbelliferyl Phosphate (MUP).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was determined following the Clinical and Laboratory Standards Institute (CLSI) Protocol EP5-A2. Two lots of AxSYM HbA1c reagents and one lot of kit calibrators and kit controls were used throughout the study. The low kit control, high kit control, and two whole blood samples were assayed in replicates of two, twice daily, for 20 days (n=80). There were at least 2 hours between each run on each of two AxSYM instruments. A calibration curve was generated on each instrument during Run 1 on Day 1 for each reagent lot. Controls and samples were run as samples every day after that. Fresh AxSYM HbA1c kit controls and samples were used for each run.

The results are summarized below.

Precision Lot 1

Low Control	Mean		Within Run		Total	
AxSYM	n	(%HbA1c)	SD	%CV	SD	%CV
Instrument 1	80	4.935	0.08	1.7	0.12	2.4
Instrument 2	80	4.876	0.09	1.8	0.12	2.5
High Control						
Instrument 1	80	9.338	0.31	3.4	0.48	5.2
Instrument 2	80	9.194	0.36	3.9	0.53	5.7
Non-diabetic sample						
Instrument 1	80	5.450	0.10	1.9	0.13	2.4
Instrument 2	80	5.320	0.13	2.4	0.17	3.3

Diabetic Sample						
Instrument 1	80	8.139	0.27	3.4	0.31	3.8
Instrument 2	80	7.936	0.24	3.0	0.32	4.0

Precision Lot 2

Low Control	Mean		Within Run		Total	
AxSYM	n	(%HbA1c)	SD	%CV	SD	%CV
Instrument 1	80	4.791	0.07	1.5	0.14	2.8
Instrument 2	80	4.803	0.10	2.1	0.14	2.8
High Control						
Instrument 1	80	9.288	0.29	3.1	0.42	4.5
Instrument 2	80	9.130	0.30	3.3	0.46	5.0
Non-diabetic sample						
Instrument 1	80	5.338	0.12	2.3	0.15	2.8
Instrument 2	80	5.431	0.14	2.6	0.2	3.7
Diabetic Sample						
Instrument 1	80	8.257	0.27	3.3	0.31	3.7
Instrument 2	80	8.309	0.32	3.9	0.37	4.5

b. Linearity/assay reportable range:

The linear range was evaluated using 5 high HbA1c whole blood sample pools that were diluted with 5 separate low HbA1c whole blood sample pools. The linear range was also evaluated using whole blood Calibrator F diluted with whole blood Calibrator A. The dilutions were made as recommended in the CLSI guideline EP6-A and an analysis was carried out in accordance with the guideline by performing 1st, 2nd and 3rd order least squares regressions. The results demonstrated linearity for all datasets. In addition, the observed values were within $\pm 10\%$ of the expected values for all samples tested. The results submitted demonstrated linearity across the claimed measuring range of 4.0% to 14.5% HbA1c.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The AxSYM HbA1c Standard Calibrators are prepared from glycated hemoglobin human whole blood diluted in non-glycated hemoglobin human whole blood and are traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The AxSYM HbA1c assay is certified with the National Glycohemoglobin Standardization Program (NGSP)/DCCT.

The AxSYM HbA1c kit controls are prepared from glycated hemoglobin human whole blood diluted in non-glycated hemoglobin human whole blood and values are assigned with the IFCC reference calibrators.

Reference Calibrator values are assigned from the IFCC reference calibrators by performing a minimum of one run on each of five AxSYM instruments over three days. The same procedure is performed using the reference calibrators to assign values to the kit calibrators.

Control values are assigned from the IFCC reference calibrators following the same instrument protocol as the calibrators.

Calibrators and controls are claimed to be stable until the expiration date printed on the label when stored at -20°C. Unopened vial stability testing was performed at the intended storage temperature of -20°C and results supported the expiration dating printed on the label. In use stability was performed with vials that were stored at -20°C, then opened and closed properly and stored at 2°-8°C. Results of this testing support the stability claims of 30 days after opening when the vials are closed properly and stored at 2°-8°C.

d. Detection limit:

The reportable range is 4 to 14.5% HbA1c (see linearity section above).

e. Analytical specificity:

Studies for interfering substances, rheumatoid factor, protein (gamma globulins), acetylsalicylate, sodium cyanate, ascorbic acid, urea, bilirubin and triglyceride were carried out according to CLSI document EP7-2A. For these studies, low and high % HbA1c fluoride oxalate whole blood sample pools representing the normal and diabetic ranges were spiked with the interfering substance. Unspiked samples for each interfering substance were also prepared from the same low and high % HbA1c sample pools by adding an equal volume of the buffer that was used to prepare the interfering substance stock solutions in place of the interfering substance. The spiked and unspiked samples were tested in the AxSYM HbA1c assay in replicates of 10. Interference was defined as a difference greater than 0.75% HbA1c between samples with and without interferant. The following concentrations were tested and did not demonstrate any interference.

Interfering Substance	Interfering Substance Concentration
Acetylsalicylate	50 mg/dL
Ascorbic Acid	50 mg/dL
Bilirubin	20 mg/dL
Gamma Globulin	5 g/dL

Interfering Substance	Interfering Substance Concentration
Rheumatoid Factor	200 IU/mL
Sodium Cyanate	50 mg/dL
Triglycerides	1600 mg/dL
Urea	667 mg/dL

A hemoglobin concentration interference study was carried out using International Federation of Clinical Chemistry (IFCC) reference controls containing different levels of Hemoglobin (7, 14 and 21 g/dL), but the same level of %HbA1c. These were tested in the AxSYM HbA1c assay and the % Interference and % HbA1c difference were calculated. Interference was defined as a difference greater than 0.75% HbA1c between samples with and without interferant. The concentrations tested did not demonstrate any interference with hemoglobin concentrations form 7 to 21 g/dL.

A pre-glycated hemoglobin study was carried out using 3 whole blood samples in the normal and diabetic range. The samples were treated with high concentrations of glucose (1400 mg/dL for 3 hours at 37° C), producing labile HbA1c spiked samples. Unspiked samples were prepared in the same manner, but were treated with an equal volume of diluent used to prepare the glucose stock solution in place of the 1400 mg/dL glucose stock solution. The spiked and unspiked samples were tested in the AxSYM HbA1c and the % Interference and % HbA1c difference were calculated. Interference was defined as a difference greater than 0.75% HbA1c between samples with and without interferant. The concentrations tested did not demonstrate any interference.

A hemoglobin (Hb) variant interference study was carried out using samples provided by the IFCC known to contain Hemoglobin variants C, D, E, F, J, and S. These were tested in the AxSYM HbA1c assay and the %HbA1c difference and % difference between the mean concentration obtained for each sample and the corresponding % HbA1c reference value provided by the IFCC was determined. The hemoglobin variants C, S, J and F each had differences within ± 0.75 of the IFCC supplied value. Therefore hemoglobin variants C, S, J and F did not interfere in the AxSYM HbA1c assay. The hemoglobin variants D and E had differences greater than ± 0.75 of the IFCC supplied value. Therefore hemoglobin variants D and E do interfere in the AxSYM HbA1c assay. A statement to this affect has been inserted in the Reagent Package Insert under the 'Limitations of the Procedure' section.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Correlation studies based on guidance the CLSI protocol EP9-A2 were performed to compare the AxSYM HbA1c assay with the Tosoh G7 Automated HPLC-HbA1c Variant Analysis device. 300 whole blood patient samples ranging in concentration from 4.4% to 14.5% HbA1c were tested with both devices. Passing-Bablok regression was performed and calculated a slope of 1.02 and a y-intercept of -0.35 with a Pearson correlation r value of 0.96.

b. *Matrix comparison:*

In order to compare anticoagulant collection tubes, ten patient samples were collected in fluoride oxalate, fluoride EDTA and potassium EDTA whole blood collection tubes. The potassium EDTA samples were subjected to one freeze thaw cycle and thawed prior to testing to minimize settling of red blood cells. All samples were tested neat and then tested again following spiking with HbA1c at three levels to cover the concentration range of the AxSYM HbA1c assay as determined by AxSYM analysis (4.9 to 14.3% HbA1c). With the exception of two spiked samples, the fluoride EDTA and potassium EDTA results were within $\pm 1\%$ HbA1c compared to the fluoride oxalate tubes. The two samples that exceeded $\pm 1\%$ had results of 10.3 in fluoride oxalate and 8.9 in potassium EDTA and 14.3 in fluoride oxalate and 13.1 in fluoride EDTA.

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Depending on the assay used, HbA1c is approximately 4% to 6% in nondiabetics, 6% to 8% in controlled diabetics, and can be as much as 20% in uncontrolled diabetics.^{3,16, 17} Diabetic patients with HbA1c levels below 7% meet the therapeutic goal of the American Diabetes Association.¹⁸ In a representative study, fluoride oxalate whole blood samples from 100 apparently healthy donors were tested using the AxSYM HbA1c assay. The median AxSYM value was 5.1% HbA1c. Using non-parametric analysis, the central 95% of the population was 4.6 to 6.0% HbA1c. It is recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending upon geographical, patient, dietary or environmental factors.

³ Goldstein DE, Little RR, Weidmeyer H-M, et al. Glycated hemoglobin:methodologies and clinical applications. Clin Chem 1986;32(10):864- 70.

¹⁶ Nathan DM, Singer DE, Hurxthal K, et al. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984;310(6): 341-6.

¹⁷ Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. Philadelphia,PA; WB Saunders; 1994:984-6.

¹⁸ Goldstein DE, Little RR, Lorenz RA, et al. American Diabetes Association. Tests of Glycemia in Diabetes. Diabetes Care 2003;26(1):St 06-8.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.