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

A. 510(k) Number:

k100304

B. Purpose for Submission:

New device

C. Measurand:

Glycosylated hemoglobin (HbA1c)

D. Type of Test:

Quantitative, immunoassay

E. Applicant:

Tosoh Bioscience, Inc.

F. Proprietary and Established Names:

ST AIA-PACK HbA1c, ST AIA-PACK HbA1c Calibrator Set, ST AIA-PACK HbA1c Pretreatment Solution, ST AIA-PACK HbA1c Control Set

G. Regulatory Information:

Device	Regulation	Class	Product Code	Panel
ST AIA-PACK HbA1c	21 CFR § 864.7470, Glycosylated hemoglobin	II	LCP	Hematology (81)
ST AIA-PACK HbA1c Calibrator Set	21 CFR § 862.1150, Calibrator	II	JIT	Chemistry (75)
ST AIA-PACK HbA1c Control Set	21 CFR § 862.1660, Quality control material, (assayed and unassayed)	I, reserved	JJY	Chemistry (75)

H. Intended Use:

1. Intended use(s):

See H.2. indications for use below.

2. Indication(s) for use:

ST AIA-PACK HbA1c is designed for *In Vitro* Diagnostic Use Only for the quantitative measurement for percent concentration of Hemoglobin A1c (HbA1c) in EDTA whole blood on Tosoh AIA System Analyzer. HbA1c measurement is used in the management and treatment of diabetes.

The ST AIA-PACK HbA1c CALIBRATOR SET is intended for *In Vitro* Diagnostic Use Only for the calibration of ST AIA-PACK HbA1c Assay.

The ST AIA-PACK HbA1c CONTROL SET is intended for *In Vitro* Diagnostic Use Only for performing quality control procedures with the ST AIA-PACK HbA1c Assay.

The ST AIA-PACK HbA1c PRETREATMENT SOLUTION is intended for *In Vitro* Diagnostic Use Only for the pretreatment of the patient samples or ST AIA-PACK HbA1c CONTROL SET for the ST AIA-PACK HbA1c assay.

3. Special conditions for use statement(s):

For prescription use

Not for use with persons with hemoglobin < 7.0 g/dL or hemoglobin F concentrations greater than 10%.

4. Special instrument requirements:

Tosoh AIA 1800 System Analyzer

I. Device Description:

The ST AIA-PACK HbA1c reagents, pretreatment solution, calibrators, and controls are in vitro diagnostic devices for use exclusively on Tosoh AIA System analyzers. The ST AIA-PACK HbA1c contains magnetic beads in test cups that are coated with polyclonal sheep antibody conjugated to bovine alkaline phosphatase. The ST AIA-PACK HbA1c CALIBRATOR SET is a set of 6 calibrators traceable to IFCC reference material. The calibrators are in ready-to-use form.
The AIA-PACK HbA1c CONTROL SET contains two levels of HbA1c. The control materials are lyophilized materials and should be reconstituted with the pretreatment solution. Both the calibrators and controls are derived from human blood. Both devices have been tested by FDA-cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled

with the same precautions as used for patient samples. ST AIA-PACK HbA1c PRETREATMENT SOLUTION contains a buffered detergent for lysing cells. All components are purchased separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Tosoh Automated Glycohemoglobin Analyzer HLC-723G7 (k011434)

2. Predicate K number(s):

   k011434

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (k011434)
Indications for Use	Same	Glycosylated hemoglobin measurements obtained by this device are used in the management and treatment of diabetes.
Cross reactivity to hemoglobin variants	Same	No significant cross reactivity to HbC & HbD

Differences		
Item	Device	Predicate (k011434)
Cross reactivity to hemoglobin variants	Some crossreactivity was shown for HbS and HbE, (see section M1e, below)	No cross reactivity with HbS, some cross-reactivity with HbE
Cross reactivity with HbF	Cross reactivity at > 10% HbF	Cross reactivity at >14% HbF
Test Method	Immuno-enzymometric assay (IMEA) using fluorescence detection	HPLC
Assay Range	3.0-14.0%	3.0-18.4%
Calibrator Set	ST AIA-PACK HbA1c Calibrator Set 6 point aligned to IFCC reference material and NGSP	Hemoglobin a1c Calibrator Set 2 point aligned to IFCC reference material and NGSP
Reference Range	4.0 to 6.0%	4.2 to 5.8%
Assay time	38 minutes (10 min. incubation time and 20 min. pretreatment)	2.2 minutes

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples

CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

HbA1c present in the pretreated test sample is captured on the magnetic beads along with hemoglobin and bound with enzyme-labeled sheep anti-HbA1c polyclonal antibody in the AIA-PACK test cup. The beads are washed to remove the unbound materials and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled polyclonal antibody that binds to the beads is directly proportional to the HbA1c concentration in the test sample. HbA1c percent concentrations are calculated from a curve generated by using the ST AIA-PACK HbA1c Calibrator Set.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision for the ST AIA-PACK HbA1c assay was evaluated using three AIA-1800 analyzers and three lots of reagents according to CLSI EP5-A2. One calibration curve was used throughout the precision study. Three levels of pooled EDTA whole blood specimens were run in duplicate twice daily for 20 days (N=80). Specimens were aliquoted and stored at -30°C and thawed once/day. Results are below:

Within Run Precision

Specimen	Reagent Set # 1			Reagent Set # 2			Reagent Set # 3		
	Mean (%)*	Pooled SD (%)*	CV (%)	Mean (%)*	Pooled SD (%)*	CV (%)	Mean (%)*	Pooled SD (%)*	CV (%)
HWB-1	5.3	0.096	1.8	5.5	0.099	1.8	5.5	0.090	1.6
HWB-2	8.1	0.093	1.1	8.2	0.137	1.7	8.2	0.122	1.5
HWB-3	13.1	0.244	1.9	13.0	0.192	1.5	13.0	0.204	1.6

Total Precision

Specimen	Reagent Set # 1			Reagent Set # 2			Reagent Set # 3		
	Mean (%)*	**Pooled SD (%)***	**CV (%)**	**Mean (%)***	**Pooled SD (%)***	**CV (%)**	**Mean (%)***	**Pooled SD (%)***	**CV (%)**
HWB-1	5.3	0.175	3.3	5.5	0.120	2.2	5.5	0.109	2.0
HWB-2	8.1	0.256	3.2	8.2	0.161	1.9	8.2	0.158	1.9
HWB-3	13.1	0.528	4.0	13.0	0.314	204	13.0	0.310	2.4

b. Linearity

A recovery study for linearity was performed according to CLSI Protocol EP6-A. 11 specimens with concentrations from 2.2%-15.3% HbA1c were assayed in triplicate using one reagent lot on one AIA 1800. The percent recoveries between the observed values and the expected values were 97.8-104.9%. The regression was: $y = 1.024x - 0.104$, $r = 0.999$.

The results of the study support the claimed reportable range of 3.0%-14.0% HbA1c.

High dose hook effect was evaluated on one AIA-1800 by analyzing the fluorescence rate of a control solution containing 25% HbA1c in triplicate with one lot of reagents. The fluorescence rate is greater than that of the highest calibrator (approximately 16% HbA1c). No hook effect was observed up to 25% HbA1c.

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

Traceability:
The ST AIA-PACK HbA1c assay has met the requirements of the National Glycohemoglobin Standardization Program (NGSP). The NGSP certification requires annual renewal. The current list of NGSP certified methods is available on the NGSP website at: http://www.ngsp.org/prog/index.html

ST AIA-PACK, HbA1c Calibrators contain 6 levels and are traceable to IFCC reference materials.

Assignment of control values:
AIA-PACK HbA1c Control Set contains Level 1 (approximately 5.5%) and Level 2 (approximately 9.5%). A master pool is diluted to pre-determined HbA1c concentrations for Level 1 and Level 2 controls. Values are assigned by analyzing the two diluted pools using multiple reagent lots and multiple analyzers. A grand mean, SD and CV are calculated from the data. The control ranges are set at ± 10% of the grand mean.

Stability, closed-vial:
Real time closed vial shelf life stability study for the AIA-PACK HbA1c assay reagents (ST AIA-PACK HbA1c, ST AIA-PACK, HbA1c Calibrator Set, AIA-PACK HbA1c Control Set, ST AIA-PACK HbA1c Pretreatment Solution) was conducted using a single AIA-1800 analyzer and four lots of each reagent (four reagent sets). All reagents were stored at 8-12° C for up to 13 months. Stability was determined to be 12 months at 8-12° C.

Stability, open vial:
Real time open vial stability testing was performed for the AIA-PACK HbA1c Control Set, Levels 1 and 2 at 10° C for 8 days and 30° C for 7 days. Recoveries of five replicates for each control were evaluated at various time points using one AIA-1800 and one reagent lot. Open vial control stability is 7 days at 10°C and 6 hours at 30°C.

Real time open vial recovery studies for stability were performed for the ST AIA-PACK HbA1c Calibrator Set at 10° C for 2 days and 30° C for 7 hours. Calibrators are stable for 1 day at 10° C and 6 hours at 30° C. Pretreated calibrators were also evaluated at the same temperature ranges. Pretreated calibrators are stable for up to 2 hours prior to analysis at 30° C and up to 1 day at 10° C.

Real time ST AIA-PACK HbA1c Pretreatment Solution stability was evaluated daily for 91 days stored at 2-8° C. The Pretreatment Solution met the sponsor's stability criteria. Stability was determined to be 90 days at 2-8° C.

The in-use open test cup real time stability study was conducted by opening the ST AIA-PACK and storing the individual test cups at 10° C for 8 days and 30° C for 2 days. At each stability time point, five replicates of three unaltered EDTA whole blood specimens and AIA-PACK HbA1c CONTROL Level 1 and Level 2 were assayed for % HbA1c using a single set of reagents on the AIA-1800. The AIA-1800 was calibrated on Day 0. Recovery data supports the sponsor's claims that reagent packs are stable for 1 day at 30° C and 7 days at 10° C.

Recovery studies were performed for sample stability before pretreatment using 3 EDTA whole blood samples stored at 30°C for 2 days, 10°C for 8 days and -20°C for 197 days. Samples are stable before pretreatment for 1 day at 30°C, 7 days at 10°C and 60 days at -20°C.

d. Detection limit:

See item M.1.b., Linearity, above.

e. Analytical specificity:

Endogenous and Exogenous Interference:

Interference was evaluated on the AIA-1800 using three whole blood samples with HbA1c concentrations of approximately 5.3%, 8.0%, and 13%. Each sample was spiked with various concentrations of interfering substance and evaluated against a sample with no interferences added. Samples were run in triplicate. Interference was defined as >10% difference in recovery between the spiked sample and the non-spiked sample. Results were as follows:

Interfering Substance	Conclusion
Bilirubin, conjugated	No interference up to 18 mg/dL conjugated bilirubin
Bilirubin, free	No interference up to 16 mg/dL free bilirubin
Lipemia	No interference up to 1666 mg/dL triglyceride
Albumin	No interference up to 50 mg/mL albumin
Ascorbic Acid	No interference up to 20 mg/mL ascorbic acid
Trisodium citrate	No interference up to 20 mg/mL trisodium citrate
EDTA – 2K	No interference up to 10 mg/mL EDTA-K2
Rheumatoid Factor	No interference up to 550 IU/mL Rh Factor

Hemoglobin Variant Interference:

Cross-reactivity to Hemoglobin Variant F was studied on ST AIA-PACK HbA1c. Specimens at varying HbF levels were prepared by mixing high (14.7% HbF) and low (0.3% HbF) l EDTA whole blood specimens to prepare a eleven samples containing 0.3 – 14.7% HbF as measured on the predicate, the Tosoh Automated Glycohemoglobin Analyzer HLC-723G7 (G-7) Variant Analysis Mode. Samples were tested in singlicate on the AIA-1800 and the G-7. Recoveries were calculated against the predicate. Recoveries ranged from (96%-104%). Interference was defined as % HbA1c recovery >10%. Up to 10% Hemoglobin F does not significantly interfere with the assay result of ST AIA-HbA1c. More than 10% Hemoglobin F does interfere with the ST AIA-HbA1c result.

EDTA whole blood specimens containing hemoglobin variants C, D, E and S were obtained from an outside NGSP certified laboratory. All specimens were analyzed for %HbA1c using the ST AIA-PACK HbA1c assay on the AIA-1800 analyzer. Samples were tested in singlicate for all of the Hb variants. The % HbA1c value from the NGSP laboratory's boronate affinity method was used as the reference and the % recovery was calculated for each specimen. Recovery of the Variant Hemoglobin C, D, S and E specimens should be within 100 ± 10 %. All interference study results are included in the labeling.

The results are summarized below.

Hb Variant	TOSOH AIA-1800 %HbA1c	Boronate affinity	% recovery
A/C	5.4	5.9	91.5
	6.7	6.8	98.5
	7.4	6.8	108.8
	7.4	7.6	97.4
	8.6	8.7	98.9
	10.8	11.0	98.2
	14.8	13.7	108.0
A/D	5.4	5.6	96.4
	6.2	6.3	98.4
	6.9	7.5	92.0
	9.7	9.4	103.2
	9.8	10.0	98.0
A/S	4.7	5.0	94.0
	6.4	6.7	95.5
	8.5	10.0	85.0
	10.6	10.2	103.9
	11.1	11.3	98.2
A/E	5.1	5.7	89.5
	6.9	7.4	93.2
	5.1	5.7	89.5
	6.4	6.8	94.1
	9.6	10.9	88.1
	10.3	10.9	94.5

There was no significant interference from HbA/C or HbA/D. One out of five variant HbS specimens was outside the criteria of ± 10% recovery. Three out of six variant HbE specimens were outside the criteria of ± 10% recovery.

Labile, Carbamyl and Acetaldehyde Hemoglobins

Three unaltered EDTA whole blood samples with HbA1c levels at low, mid and high levels were spiked with glucose up to1000 mg/dL. Similarly, three unaltered EDTA whole blood samples with HbA1c levels at low, mid and high were spiked with sodium cyanate up to 50 mg/dL or acetaldehyde up to 50 mg/dL. The specimens were incubated at 37° C for 4 hours and then assayed with ST AIA-PACK HbA1c in triplicate. The mean was calculated.

The specimen value with no added glucose or sodium cyanate or acetaldehyde was used as the reference value. No interference was defined as recovery of 100% ± 10%. There was no interference from sodium cyanate or acetaldehyde up to 50 mg/dL, nor glucose up to 1000 mg/dL.

Effect of Low Hemoglobin

90 whole blood samples with hemoglobin concentrations ranging from 6.9-16 g/dL and HbA1c concentrations from 4.4-12.95% were evaluated for recovery by the predicate and the AIA 1800. Recoveries ranged from 87.8% to 106.3%. The sponsor has the following statement in the labeling "Hemoglobin levels less than 7.0 g/dL yielded falsely decreased HbA1c results."

f. Assay cut-off:

Not applicable.

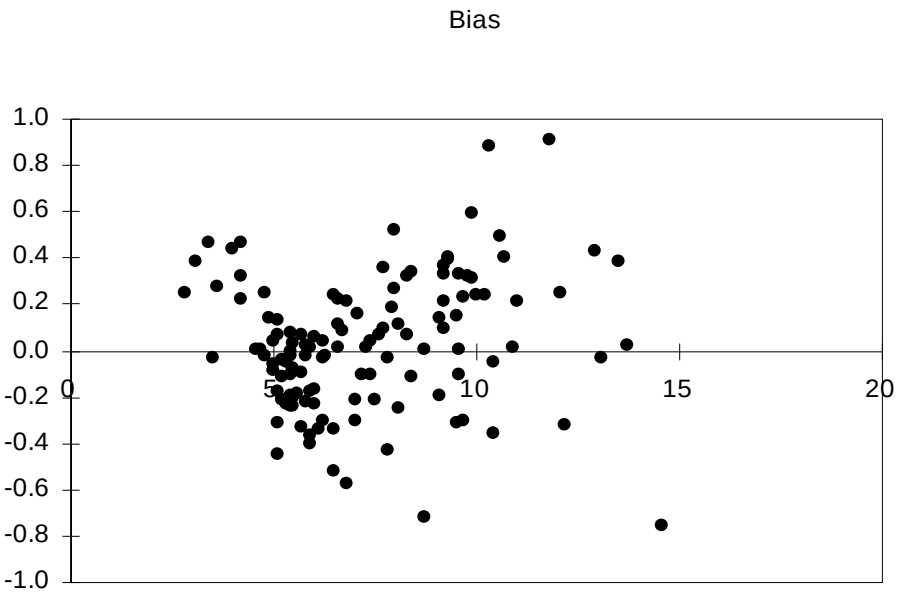
2. Comparison studies:

a. Method comparison with predicate device:

126 EDTA whole blood specimens were assayed in singlicate utilizing the ST AIA-PACK HbA1c assay on the AIA-1800 analyzer and the Tosoh G-7 Variant Analysis Mode at a single site following CLSI EP9-A2. HbA1c values ranged from 3.0% to 13.9% HbA1c. Both Deming regression and linear regression analyses were performed. Results are summarized below:

	Deming Regression (95% CI)	Linear Regression
Slope	1.027 (1.006, 1.047)	1.020 (1.000, 1.040)
Intercept	-0.168 (-0.326, -0.011)	-0.120 (-0.277, -0.037)
R	0.99	0.99

The sponsor provided a bias plot for the data.



b. Matrix comparison:

This device is for EDTA whole blood only.

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:
The American Diabetes Association (ADA) expected values range is 4.0 to 6.0 % for people without diabetes[1-4].

[1]Parnes B., Niebauer L., Holcomb S., Dichinson M., Vanvorst B., Pace W., Provider Deferred Decisions on Hemoglobin A1c Report from the Colorado Research Network (CaR) the High Plains Research Network (HPRN). *J. Am. Fam. Med*., **19**(1); 20-23 (2006).
[2] American Diabetes Association Position Statement, Test of Glycemia in Diabetes. *Diabetes Care*, **27**(Suppl 1); 91-93 (2004).
[3] Little R. R., Rohlfing C., Wiedmeyer H. M., Myers G. L., Sacks B. D., Goldstein D. E., The National Glycohemoglobin Standardization Program (NGSP): a five year progress report. *Clin. Chem*., **47**; 1985-1992 (2001).
[4] Rohlfing C. L., Wiedmeyer H. M., Little R. R., England J. D., Tennill A., Goldstein D. E. Defining the relationship between plasma glucose and HbA1c: analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. *Diabetes Care*, **25**; 275-278 (2002).

The sponsor used CLSI C28-A3 to verify the expected range values with a 120 sample study from non-diabetic patients and obtained a range of 3.8-6.0%.

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.